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(54) **Title**: USE OF PEI FOR THE IMPROVEMENT OF ENDOSOMAL RELEASE AND EXPRESSION OF TRANSFECTED NUCLEIC ACIDS, COMPLEXED WITH CATIONIC OR POLYCATIONIC COMPOUNDS

(57) **Abstract**: The present invention is directed to complexed precomplexed nucleic acids, preferably mRNAs, which have been precomplexed in a first step with PEI in an N/P ratio between 0.05 and 2, preferably in an N/P ratio between 0.1 and 1, and which have been further complexed in a second step with a cationic compound. The present invention is furthermore directed to the use of such complexed precomplexed nucleic acids for the improvement of the endosomal release of nucleic acids and optionally the improvement of expression of an encoded protein or peptide. It is also directed to compositions comprising such complexed precomplexed nucleic acids and to the use of such complexed precomplexed nucleic acids or compositions thereof for gene therapy and/or the treatment of various diseases as mentioned herein, e.g. by vaccination. The present invention is also directed to methods for preparing and administering these complexed precomplexed nucleic acids or compositions thereof and to kits, comprising these complexed precomplexed nucleic acids or compositions thereof.

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Use of PEI for the improvement of endosomal release and expression of transfected nucleic acids, complexed with cationic or polycationic compounds

The present invention is directed to complexed precomplexed nucleic acids, preferably mRNAs, which have been precomplexed in a first step with PEI in an N/P ratio between 0.05 and 2, preferably in an N/P ratio between 0.1 and 1, and which have been further complexed in a second step with a cationic compound. The present invention is furthermore directed to the use of such complexed precomplexed nucleic acids for the improvement of the endosomal release of nucleic acids and optionally the improvement of expression of an encoded protein or peptide. It is also directed to compositions comprising such complexed precomplexed nucleic acids and to the use of such complexed precomplexed nucleic acids or compositions thereof for gene therapy and/or the treatment of various diseases as mentioned herein, e.g. by vaccination. The present invention is also directed to methods for preparing and administering these complexed precomplexed nucleic acids or compositions thereof and to kits, comprising these complexed precomplexed nucleic acids or compositions thereof.

Various diseases today require a treatment which involves administration of peptide-, protein-, and nucleic acid-based drugs, particularly the transfection of nucleic acids into cells or tissues. The full therapeutic potential of peptide-, protein-, and nucleic acid-based drugs is frequently compromised by their limited ability to cross the plasma membrane of mammalian cells, resulting in poor cellular access and inadequate therapeutic efficacy. Today this hurdle represents a major challenge for the biomedical development and commercial success of many biopharmaceuticals (see e.g. Foerg and Merkle, Journal of Pharmaceutical Sciences, published online at www.interscience.wiley.com, DOI 10.1002/jps.21117).

For some diseases or disorders, gene therapeutic approaches have been developed as a specific form of such treatments. These treatments in general utilize transfection of nucleic acids or genes into cells or tissues, whereas gene therapeutic approaches additionally involve the insertion of one or more of these nucleic acids or genes into an individual's cells and tissues to treat a disease, e.g. hereditary diseases, in which a defective mutant allele is replaced with a functional one.

Transfection of nucleic acids into cells or tissue is, however, not simple and typically dependent on many factors. For successful delivery, e.g., delivery of nucleic acids or genes into cells or tissue, many barriers must be overcome. Presently there are four barriers that must be overcome by transfection vectors to achieve successful gene delivery. Particularly, the vector must be able 1) to tightly compact and to protect the nucleic acid, e.g., to stabilize the nucleic acid and prevent it from early degradation, 2) to target specific cell-surface receptors, 3) to disrupt the endosomal membrane, and 4) to deliver the cargo, e.g. the nucleic acid or gene, into the nucleus or the cytoplasm. These four goals may be achieved by using a combination of different compounds or vectors. However, there are also some compounds or vectors, which overcome at least some of these barriers.

Most usually, transfection, e.g. of nucleic acids, is carried out using viral or non-viral vectors. For successful delivery, these viral or non-viral vectors must be able to overcome the above mentioned barriers. The most successful gene therapy strategies available today rely on the use of viral vectors, such as adenoviruses, adeno-associated viruses, retroviruses, and herpes viruses. However, despite their success, there are many problems associated with viruses related to immunogenicity, cytotoxicity, and insertional mutagenesis. A solution to this problem may be found in the use of non-viral vectors. Although non-viral vectors are not as efficient as viral vectors, many non-viral vectors have been developed to provide a safer alternative in gene therapy. Some of the most common non-viral vectors include (positively charged) polymers for transfection, calcium phosphate, lipids, proteins or peptides, dendrimers, or other polymers. Particularly cationic compounds such as positively charged cationic polymers, cationic lipids, etc., glycerol based cationic lipids, allow an efficient transfection of nucleic acids, due to their superior condensing properties.

In this context, cationic polymers may include, e.g., DEAE-dextran, poly-L-lysine, modified polyaminoacids, such as β-aminoacid-polymers, or reversed polyamides, modified polyethylenes, such as poly(N-ethyl-4-vinylpyridinium bromide) (PVP), modified acrylates, such as poly(dimethylaminoethyl methylacrylate) (pDMAEMA), modified amidoamines, such as poly(amidoamine) (pAMAM), modified polybetaaminoesters (PBAE), such as diamine end modified 1,4 butanediol diacrylate-co-5-amino-1-pentanol polymers, dendrimers, such as polypropylamine dendrimers or pAMAM based dendrimers, polyimine, such as poly(ethyleneimine) (PEI), or poly(propyleneimine), polyallylamine, sugar backbone based polymers, such as cyclodextrin based polymers, dextran based polymers or chitosan, silan backbone based polymers, such as PMOXA-PDMS copolymers, or blockpolymers consisting of a combination of one or more cationic polymers (blocks) as mentioned above and of one or more hydrophilic- or hydrophobic polymers (blocks) (e.g polyethyleneglycole) (see e.g. Smedt *et al.*, Pharmaceutical Research, Vol. 17, No. 2, 2000).

Cationic polymers may furthermore include cationic lipids, e.g., glycerol-based cationic lipids, non-glycerol-based cationic lipids, or cholesterol-based cationic lipids, etc. Cationic lipids may include, e.g., DOPC, DODAP, Dioleyl phosphatidylethanol-amine (DOPE), Dioleoxypropyltrimethylammonium chloride (DOTMA), DC-Chol, DOSPA, DODAB, DOIC, DMEPC, Dioctadecylamidoglicylspermin (DOGS), Dimyristo-oxypropyl dimethyl hydroxyethyl ammonium bromide (DIMRI), DOTAP: dioleoyloxy-3-(trimethylammonio)propane, O,O-ditetradecanoyl-N-(α-trimethylammonioacetyl) diethanolamine chloride (DC-6-14), rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride (CLIP1), rac-[2(2,3-dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium (CLIP6), and rac-[2(2,3-dihexadecyloxypropyl-oxysuccinyloxy)ethyl]-trimethylammonium (CLIP9) (see Zhdanov *et al.* Biochemistry 58 (2002) 53-64). Glycerol-based cationic lipids may include, e.g., DOTMA: [1-(2,3-sioleyloxy)propyl)]-N,N,N-trimethylammonium chloride, DOTAP, DMRIE, SOSPA, non-glycerol based cationic lipids may include, e.g., DOGS, di-C14-amidine, DOTIM, SAINT, and cholesterol-based cationic lipids may include, e.g., DC-Chol, BGTC, and CTAP, etc.

Among these non-viral vectors, peptide-based vectors are particularly advantageous over other non-viral strategies in that they are more or less capable to achieve all of the above mentioned four goals. For example, cationic peptides rich in basic residues such as lysine

and/or arginine are able to efficiently condense nucleic acids into small, compact particles that can be stabilized in serum. Furthermore, a peptide ligand may be attached to the polyplex structure, which allows targeting of the complex to specific receptors and/or specific cell types. Peptide sequences derived from protein transduction domains (PTDs) are in general capable to selectively lyse the endosomal membrane in its acidic environment leading to cytoplasmic release of the polyplex. Finally, short peptide sequences taken from longer viral proteins can provide nuclear localization of condensates once they are in the cytoplasm (see Martin and Rice, The AAPS Journal 2007; 9 (1) Article 3).

Further types of (cationic) peptides, which are generally cationic in nature and able to interact with plasmid DNA or other nucleic acids through electrostatic interactions may also include so called cell penetrating peptides (CPPs). CPPs are typically derived from viral, insect or mammalia proteins endowed with membrane translocation properties and include, e.g. penetratin; peptides derived from the family of HIV-1 TAT proteins or peptides; pVEC; VP22 (HSV VP22 (Herpes simplex)); MAP; KALA; PpTG20; Proline-rich peptides; chimeric peptides, including transportan or proteins or peptides from the MPG familiy, e.g. MPG-peptides, such as Pβ or Pα; peptides of the penetratin family, such as Antennapedia (Drosophila antennapedia) derived peptides, e.g. pAntp or pIsl; Pep-1; L-oligomers; arginine-rich peptides, e.g. oligoarginines; calcitonin-peptides; FGF; lactoferrin; Pep-1, antimicrobial-derived CPPs, such as Buforin-2, Bac 715-24, or SynB; Pvec; hCT-derived peptides, such as hCT(9-32) or hCT(9-32)-br; SAP, protamine, poly-L-Lysine, poly-Arginine, histones, spermine, spermidine, etc. CPPs typically represent short peptide sequences of 10 to about 30 amino acids which can cross the plasma membrane of mammalian cells and may thus offer unprecedented opportunities for cellular drug delivery. In fact, in a widely recognized landmark study in mice, the intraperitoneal injection of a fusion protein conjugated to Tat(47–57), an oligocationic CPP derived from human immunodeficiency virus (HIV) Tat protein, was found to let the ligated protein, b-galactosidase, overcome numerous biological barriers, distribute into virtually every organ and even pass the blood brain barrier (see Schwarze *et al.*, Science 285:1569–1572). Nevertheless, the biomedical promise of CPPs is still far from clinical implementation.

The cationic polymers and peptides as defined above as well as the cationic compounds in general as mentioned are typically used for transfection purposes by formulating the nucleic acid or gene to be transfected prior to transfection. Upon such formulation, typically a complex is formed between the nucleic acid or gene to be transfected and the cationic compound. The complex is usually a very strong binding complex formed between the positively charged cationic compound and the negatively charged nucleic acid or gene. For the purposes of transfection, such a tight binding is advantageous since it allows a good condensation of the nucleic acid or gene prior to transfection, a compact and strong binding does not necessarily allow a good endosomal escape of the complexed nucleic acid or gene and, in some cases, may even lead to a poor or diminished release of the nucleic acid or gene in the cell and thus to a poor expression.

There are many examples in the art, utilizing such cationic compounds as defined above, particularly cationic polymers, for transfection purposes of nucleic acids, e.g. in *in vitro* approaches or clinical applications, by formulating the nucleic acid or gene to be transfected with the cationic compound. As an example, Neu *et al.* (Neu *et al.*, *J Gene Med* 2005; 7; 992-1009) discuss clinical application of synthetic polycations for gene delivery. According to Neu *et al.* (2005, supra) a successful clinical application of synthetic polycations for gene delivery primarily depends on three factors, namely an enhancement of the transfection efficiency, a reduction in toxicity and an ability of the vectors to overcome numerous biological barriers after systemic or local administration.

A review from Gao *et al.* (Gao *et al.* The AAPS Journal 2007; 9(1) Article 9) summarized methods for non-viral gene delivery and, *inter alia*, discussed cationic lipid mediated gene delivery and cationic polymer-mediated gene transfer. As discussed in Gao *et al.* (2007, supra) some cationic lipids alone exhibit good transfection activity, however, they are often formulated with a noncharged phospholipid or cholesterol as a helper lipid to form liposomes. Upon mixing with cationic liposomes, plasmid DNA is spontaneously condensed into small quasi-stable particles called lipoplexes. Advantageously, the DNA in these lipoplexes is well protected from nuclease degradation. Lipoplexes are furthermore able to trigger cellular uptake and facilitate the release of DNA from the intracellular vesicles before reaching destructive lysosomal compartments. A further possibility discussed in Gao *et al.* (2007, supra), the use of cationic polymer-mediated gene transfer, involves

cationic polymers such as polyethylenimine (PEI), polyamdidoamine and polypropylamine dendrimers, polyallylamine, cationic dextran, chitosan, cationic proteins (polylysine, protamine, histones) and cationic peptides. Among these cationic polymers, PEI is regarded as the most active and most studied polymer for gene delivery. According to Gao *et al.* (2007, supra), DNA-to-PEI ratios, the molecular weight and configuration of PEI, as well as the concentration of DNA and polymer and the ionic strength of the solvent for preparation are important factors for PEI-mediated transfection that determine the physical properties of the DNA/PEI complexes (polyplexes) and their transfection activity.

Among the polycations presently used for gene delivery, poly(ethylene imine) PEI takes a prominent position, due to its potential for endosomal escape. PEI has a high cationic charge density due to its nitrogen atoms which are capable of protonation. In the endosome PEI becomes protonated and thereby can buffer endosomal pH and thereby induces the rupture of the endosomal membrane by osmotic swelling (Sonawane *et al.* 2003, J. Biol. Chem. 2003, Nov 7, 278 (45): 44826-31). This hypothesis is called proton sponge. PEI is available in a broad range of molecular weights, from <1,000 Da to 1,600 kDa. In this context, it is commonly believed that the molecular weight of PEI most suitable for gene transfer ranges between 5 and 25 kDa, which can already be regarded as a high molecular weight PEI. An N/P ratio of about 2-3 is typically used in the art since approximately 90% of its charged groups must be neutralized to condense DNA (see Neu *et al.*, 2005, supra). Such an N/P ratio is therefore believed to be necessary to achieve stable complexes using branched or linear PEI. In this context, an N/P ratio is a specific determinant indicating the ratio of nucleic acid to peptide contained in such a complex on the basis of the nitrogen/phosphate ratio of all atoms involved.

However, one drawback in the use of PEI as a transfection reagent relates to its nonbiodegradable and toxic nature. It is known, that transfection of DNA with PEI in a N/P ratio of 5 induces apoptosis in Hek293 cells (Lee et al., *Bull. Korean Chem. Soc.* 2007, Vol. 28, No. 1, pp. 95-98). It is also known that the toxicity and transfection activity of PEI is dependent on its molecular weight. The most active PEI from a commercial source is 25 kDa for BPEI (branched PEI) and 22 kDa for LPEI (linear PEI). PEI with a molecular weight larger than 25 kDa is also active but exhibits greater toxicity. Such increased cytotoxicity is presumably due to aggregation of huge clusters of the cationic polymer PEI on the outer cell

membrane, which thereby induces necrosis. BPEI of 5 to 10 kDa appears to be more active in transfection and less toxic when compared with a standard "benchmark" of 25 kDa BPEI. PEI of 2 kDa or smaller is relatively nontoxic but not active in transfection. Treatment of low-molecular weight PEI with several bifunctional cross-linking reagents generates PEI oligomers that are transfectionally active. Cross-linking of small PEI with a biodegradable bond such as a disulfide or ester bond resulted in oligomers that were as active as 25 kDa PEI but significantly less toxic to cells (see Gao *et al.* 2007, supra).

PEI may be also combined with cationic polymers. Hattori *et al.* (Hattori *et al.* Biol. Pharm. Bull 30(9) 1773-1778 (2007)) disclosed that both PEI but also cationic nanoparticles have been widely used for non-viral DNA transfection. The authors previously reported that cationic nanoparticles, termed NP-OH and being composed of cholesteryl-3-beta-carboxyamidoethylene-N-hydroxyethylamine and Tween 80, could deliver plasmid DNA (pDNA) with high transfection efficiency. To increase the transfection activity of NP-OH, Hattori *et al.* (2007, supra) investigated the potential synergism of PEI and NP-OH for the transfection of DNA into human prostate tumor PC-3 cells, human cervices tumor HeLa cells and human lung adenocarcinoma A549 cells. Unfortunately, the transfection efficiency with low-molecular PEI (MW 600) was low, but a combination of NP-OH and PEI showed a better transfection rate than NP-OH alone, being comparable to commercially available lipofectamine 2,000 and lipofectamine LTX, with very low cytotoxicity. Hattori *et al.* (2007, supra) further showed, that low-molecular weight PEI could not compact pDNA in size, but rather might help to dissociate pDNA from the complex with NP-OH and release pDNA from the endosome to cytoplasm by an effect termed proton sponge effect. Accordingly, a combination of cationic cholesterol-based nanoparticles and low-molecular PEI was assumed to have potential as a non-viral DNA vector for gene delivery. The PEI used in these experiments was a very low molecular PEI having a molecular weight of about 600 and 1800 Da. The N/P ratios (nitrogen/phosphate ratio) used in these experiments were at least 1, typically at least 1 to about 3. Despite this success to significantly enhance transfection efficiency of the cationic lipid, the addition of PEI to a cationic lipid in the experiments of Hattori *et al.* (2007, supra) did not lead to a significantly improved expression of the encoded protein *in vivo*, probably due to a less efficient or low endosomal release.

Nguyen *et al.* (Nguyen *et al.*, Biomaterials. 2008 Jun; 29(18); 2783-93; Epub 2008 Apr 9) also investigated the effect of cationic polymer PEI on transfection processes, particularly the enhancement of the delivery of DNA vaccines using poly(orthoester) (POE) microspheres by blending these poly(orthoester) (POE) microspheres with PEI. Polyorthoesters (POEs) were first investigated in the 1970s by Alza Corp. (Palo Alto, CA) and SRI International (Menlo Park, CA) in a search for new synthetic biodegradable polymers for drug delivery applications. Polyorthoesters (POEs) are hydrophobic, with hydrolytic linkages that are acid-sensitive but stable to base. Microspheres formed from poly(orthoester) (POE) have been shown previously to possess certain advantages for the *in vivo* delivery of DNA vaccines. In their article, Nguyen *et al.* (2008, supra) reported on an *in vitro* characterization of POE microspheres blended by addition of poly(ethylenimine) (PEI) into the POE matrix and their enhanced efficacy. As a result, blending of a small amount of PEI (approximately 0.04 wt%, showing an N/P ratio of about 7.7:1) with POE already caused large alterations in the POE microsphere properties. Results of confocal microscopy of labeled PEI and DNA plasmids furthermore revealed that PEI caused a surface-localizing distribution of DNA and PEI within the POE microsphere as well as a focal co-localization of PEI with DNA. Nguyen *et al.* (2008, supra) showed that upon degradation, the PEI blended POE microspheres released electrostatic complexes of DNA and PEI, which were assumed to be responsible for an enhanced gene transfection rate. Blending PEI into the POE microsphere induced 50-60% greater phenotypic maturation and activation of bone marrow-derived dendritic cells in vitro, judged by the up-regulation of co-stimulatory markers on the cell surface. According to Nguyen *et al.* (2008, supra) PEI provided a better control over the rate of pH-triggered DNA release by doubling the total release time of plasmid DNA. PEI furthermore enhanced gene transfection efficiency of the microspheres up to 50-fold without exhibiting a significant cytotoxicity. Physically blending PEI with POE was therefore assumed to be a simple approach for modulating the properties of biodegradable microspheres in terms of gene transfection efficiency and DNA release kinetics. Therefore PEI was used in the appreciate amount known in the art (N/P of 7.7) to increase the gene transfer of a plasmid DNA containing uncharged degradable POE microsphere. This means, that in principle they compared the transfection of naked pDNA to the transfection of a PEI complexed pDNA, which is fairly known in the art. The authors lack to compare the pDNA/PEI formulation to the PEI blended microspheres, therefore they

show no increased expression of the blended microspheres compared to PEI complexes alone.

Summarizing the above, there are many cationic compounds known in the art, particularly cationic polymers and peptides, which are suitable to efficiently condense the DNA or gene to be transfected and to allow high transfection rates. Addition of PEI or use of PEI to this formulation furthermore allows to a certain extent a modification and in part improvement of gene transfection efficiency and DNA release kinetics. Summarizing the above, there are many cationic compounds known in the art, particularly cationic polymers and peptides, which are suitable to efficiently condense the DNA or gene to be transfected and allow to high transfection rates. However, even though high transfection rates may already be achieved using cationic polymers and peptides, their efficacy or applicability is hampered by low endosomal escape (e.g. peptides), by strong cytotoxic effects (PEI) or by low release of the nucleic acid or gene in the cytosol, due to the strong binding between the positively charged cationic compound and the negatively charged nucleic acid or gene. Accordingly, a combination of the superior endosomal effects of PEI with the favourably complex building properties and toxic attributes of other cationic polymers was not yet been shown in the art.

The object underlying the present invention is therefore to provide a composition, particularly a nucleic acid composition, and tools or methods, which both lead to an efficient gene transfection and an efficient DNA release kinetics, and additionally to a good expression of the encoded protein in the cell, preferably *in vivo* and *in vitro* at low toxicities.

This object is solved by the subject matter of the present invention, preferably by the subject matter of the attached claims. Particularly, a first aspect of the present invention solves the above object by at least one "complexed precomplexed nucleic acid", preferably at least one complexed precomplexed mRNA, wherein a nucleic acid (molecule) has been precomplexed in a first step with PEI in an N/P ratio between 0.05 and 2, and the precomplexed nucleic acid (molecule) has been further complexed in a second step with a cationic or polycationic compound.

In the context of the present invention, the term "complexed precomplexed nucleic acid" refers to a nucleic acid (molecule), preferably to an mRNA, which is precomplexed according to a first step of the present invention with PEI in the above mentioned ratio and then complexed according to a second step of the present invention with a cationic or polycationic compound, preferably in a ratio as defined herein. As surprisingly found by the inventors, the "complexed precomplexed nucleic acid" according to the present invention is particularly suitable for both the improvement of the endosomal release of nucleic acids and improvement of expression of the encoded proteins. These effects may be in part due to the potential of PEI for endosomal escape as already discussed above in the context of the proton sponge effect. PEI has a high cationic charge density due to its nitrogen atoms which are capable of protonation. In the endosome PEI becomes protonated and thereby can buffer endosomal pH. Thereby, PEI induces the rupture of the endosomal membrane by osmotic swelling (Sonawane *et al.* 2003, supra). However, PEI alone or mixed or blended with a cationic polymer is not suitable to improve expression of a nucleic acid encoded protein. As surprisingly found by the inventors of the present invention both the endosomal escape of nucleic acids and the expression of nucleic acid encoded proteins is significantly improved by the inventive stepwise treatment of the nucleic acid, i.e. precomplexing the nucleic acid in a defined ratio with (a high-molecular) PEI, in which PEI is not able to act as an transfection agent, and complexation of the already precomplexed nucleic acid with a cationic or polycationic compound in a second step. Only this "complexed precomplexed nucleic acid", leads to both an efficient endosomal escape and an efficient release of the nucleic acid from the cationic or polycationic compound within the cell. Only upon this efficient release of the nucleic acid from the cationic or polycationic compound an efficient expression of the encoded protein or peptide sequence is possible. Any of the prior art documents involve PEI together with cationic or polycationic compounds but mix or blend these components prior to complexing the nucleic acid with the mix instead of precomplexing the nucleic acid with PEI and complexing the precomplexed nucleic acid thereafter with a cationic or polycationic compound.

According to the first step of the preparation of the at least one "complexed precomplexed nucleic acid" according to the present invention, the nucleic acid (molecule) used according is precomplexed with PEI as defined above, preferably in an N/P ratio between

0.05 and 2. More preferably, the nucleic acid (molecule) used according is precomplexed with PEI as defined above in an N/P ratio between 0.05 and 1, even more preferably in an N/P ratio between 0.1 and 1. In this context, an N/P ratio is a specific determinant indicating the ratio of a nucleic acid to a peptide or a further polymer on the basis of the nitrogen/phosphate ratio of all atoms involved. In the particular context of the precomplexation of the nucleic acid (molecule) with PEI, the N/P ratio is determined as the ratio between the nucleic acid and the cationic polymer PEI used for precomplexation on the basis of the nitrogen/phosphate ratio of all atoms involved.

In the first step of the preparation of the at least one "complexed precomplexed nucleic acid" according to the present invention the nucleic acid (molecule) is precomplexed with PEI. In the context of the present invention PEI is polyethyleneimine, a polymer consisting of monomeric ethyleneimine units, wherein the polymer may be linear or branched. PEI is available in a broad molar range of molecular weights, from <1,000 Da to 1,600 kDa. A PEI suitable for the inventive purpose is preferably selected from a linear polyethyleneimine (LPEI) or a branched polyethleneimine (BPEI). Furthermore, PEI suitable for the inventive purpose is preferably selected from a high-molecular weight polyethyleneimine (high-molecular PEI). For the purpose of the present invention, a high-molecular PEI is preferably selected from a high-molecular linear polyethyleneimine (LPEI) or a high-molecular branched polyethleneimine (BPEI), having an average molecular weight of about 1 to about 1,600 kDa, more preferably selected from a high-molecular LPEI or BPEI having an average molecular weight of about 5 to about 1.500 kDa, even more preferably selected from a high-molecular LPEI or BPEI having an average molecular weight of about 10 to about 1.000 kDa, e.g. a high-molecular LPEI or BPEI having an average molecular weight of about 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or about 1.000 kDa, or may be selected from a high-molecular LPEI or BPEI of a molecular range formed by two of any of the values as mentioned above. Even though PEI with a molecular weight larger than 25 kDa, if applied in larger amounts, may exhibit toxic effects rather than low-molecular PEI, the high-molecular PEI used according to the present invention is used in an extremely small amount, which does not induce necrosis or exhibits any toxicity in the transfected cells. Additionally, a high-molecular PEI exhibits a better transfection efficiency than a low-molecular PEI.

According to a second step of the preparation of the at least one "complexed precomplexed nucleic acid" according to the present invention, the at least one precomplexed nucleic acid (molecule), prepared according to a first step as described above, is further complexed with a cationic or polycationic compound, preferably a cationic peptide. Preferably, the at least one precomplexed nucleic acid (molecule), prepared according to a first step as described above, is further complexed with the cationic or polycationic compound, preferably a cationic peptide, in an N/P ratio of about about 0.1-50, preferably in a range of about 0.5-50, even more preferably in the range of about 10-50 and most preferably in the range of about 25-50 of RNA:cationic or polycationic polymer, alternatively in a range of about 0.75-25 or 1-25.

In the context of the present invention, a cationic or polycationic compound as used in the second step of the preparation of the at least one "complexed precomplexed nucleic acid" may be selected from any cationic or polycationic compound. In the context of the present invention, a cationic or polycationic compound is preferably selected from any cationic or polycationic compound, suitable for complexing and thereby stabilizing the above defined at least one precomplexed nucleic acid, particularly an mRNA, e.g. by associating the at least one precomplexed nucleic acid with the cationic or polycationic compound. Particularly preferred, cationic or polycationic compounds are selected from cationic or polycationic peptides or proteins, including protamine, nucleoline, spermine or spermidine, or other cationic peptides or proteins, such as poly-L-lysine (PLL), poly-arginine, basic polypeptides, cell penetrating peptides (CPPs), including HIV-binding peptides, Tat, HIV-1 Tat (HIV), Tat-derived peptides, penetratin, VP22 derived or analog peptides, HSV VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs, PpT620, prolin-rich peptides, arginine-rich peptides, lysine-rich peptides, MPG-peptide(s), Pep-1, L-oligomers, Calcitonin peptide(s), Antennapedia-derived peptides (particularly from *Drosophila antennapedia*), pAntp, pIsl, FGF, lactoferrin, transportan, buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, or histones. Additionally, preferred cationic or polycationic proteins or peptides may be selected from following proteins or peptides having the following total formula: $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$, wherein l + m + n +o + x = 8-15, and l, m, n or o independently of each other may be any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, provided that the overall content of Arg,

Lys, His and Orn represents at least 50% of all amino acids of the oligopeptide; and Xaa may be any amino acid selected from native (= naturally occurring) or non-native amino acids except of Arg, Lys, His or Orn; and x may be any number selected from 0, 1, 2, 3 or 4, provided, that the overall content of Xaa does not exceed 50 % of all amino acids of the oligopeptide. Particularly preferred peptides of this formula are oligoarginines e.g. Arg_7, Arg_8, Arg_9, Arg_7, H_3R_9, R_9H_3, $H_3R_9H_3$, $YSSR_9SSY$, $(RKH)_4$, $Y(RKH)_2R$, etc. Further preferred cationic or polycationic compounds, which can be used for complexing the at least one precomplexed nucleic acid (molecule) as defined above may include cationic polysaccharides, for example chitosan, polybrene, cationic polymers, cationic lipids, e.g. [1-(2,3-sioleyloxy)propyl)]-N,N,N-trimethylammonium chloride (DOTMA), DMRIE, di-C14-amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, dioleyl phosphatidylethanol-amine (DOPE), DOSPA, DODAB, DOIC, DMEPC, dioctadecylamidoglicylspermin (DOGS), dimyristo-oxypropyl dimethyl hydroxyethyl ammonium bromide (DIMRI), dioleoyloxy-3-(trimethylammonio)propane DOTAP, O,O-ditetradecanoyl-N-(α-trimethylammonioacetyl)diethanolamine chloride (DC-6-14), rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride (CLIP1), rac-[2(2,3-dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium (CLIP6), rac-[2(2,3-dihexadecyloxypropyl-oxysuccinyloxy)ethyl]-trimethylammonium (CLIP9), oligofectamine, or cationic or polycationic polymers, e.g. modified polyaminoacids, such as β-aminoacid-polymers or reversed polyamides, etc., modified polyethylenes, such as PVP (poly(N-ethyl-4-vinylpyridinium bromide)), etc., modified acrylates, such as pDMAEMA (poly(dimethylaminoethyl methylacrylate)), etc., modified amidoamines such as pAMAM (poly(amidoamine)), etc., modified polybetaaminoester (PBAE), such as diamine end modified 1,4 butanediol diacrylate-co-5-amino-1-pentanol polymers, etc., dendrimers, such as polypropylamine dendrimers or pAMAM based dendrimers, etc., poly(propyleneimine), polyallylamine, sugar backbone based polymers, such as cyclodextrin based polymers, dextran based polymers, chitosan, etc., silan backbone based polymers, such as PMOXA-PDMS copolymers, etc., blockpolymers consisting of a combination of one or more cationic blocks (e.g. selected of a cationic polymer as mentioned above) and of one or more hydrophilic- or hydrophobic blocks (e.g polyethyleneglycole); etc. Association or complexing the at least one precomplexed nucleic acid with a cationic or polycationic compound preferably confers a stabilizing effect to the at least one precomplexed nucleic acid additional to enhanced transfection properties of the resulting complexed

precomplexed nucleic acid. The procedure for association or complexing the at least one precomplexed nucleic acid described in EP-A-1083232, the disclosure of which is incorporated by reference into the present invention in its entirety. Particularly preferred as cationic or polycationic compounds are compounds selected from the group consisting of protamine, nucleoline, spermine, spermidine, oligoarginines as defined above, such as Arg_7, Arg_8, Arg_9, Arg_7, H_3R_9, R_9H_3, $H_3R_9H_3$, $YSSR_9SSY$, $(RKH)_4$, $Y(RKH)_2R$, etc.

According to one embodiment, the nucleic acid (molecule) used for preparation of the at least one "complexed precomplexed nucleic acid" according to the present invention is preferably selected from any nucleic acid, more preferably selected from any nucleic acid suitable to encode a(t least one) peptide or protein, i.e. a coding nucleic acid, e.g. a coding DNA, selected e.g. from genomic DNA, cDNA, DNA oligonucleotides, or a coding RNA, selected e.g. from (short) RNA oligonucleotides, messenger RNA (mRNA), etc. In the context of the present invention, an mRNA is typically an RNA, which is composed of several structural elements, e.g. an optional 5'-UTR region, an upstream positioned ribosomal binding site followed by a coding region, an optional 3'-UTR region, which may be followed by a poly-A tail (and/or a poly-C-tail). An mRNA may occur as a mono-, di-, or even multicistronic RNA, i.e. an RNA which carries the coding sequences of one, two or more proteins or peptides. Such coding sequences in di-, or even multicistronic mRNA may be separated by at least one IRES sequence, e.g. as defined herein. The at least one "complexed precomplexed nucleic acid" according to the present invention may also be a ribosomal RNA (rRNA), a transfer RNA (tRNA), or a viral RNA (vRNA). Furthermore, the at least one "complexed precomplexed nucleic acid" according to the present invention may be a circular or linear nucleic acid, preferably a linear nucleic acid. Additionally, the at least one "complexed precomplexed nucleic acid" according to the present invention may be a single- or a double-stranded nucleic acid (which may also be regarded as a nucleic acid within the above meaning due to non-covalent association of two single-stranded nucleic acids) or a partially double-stranded or partially single stranded nucleic acid, which are at least partially self complementary (both of these partially double-stranded or partially single stranded nucleic acids are typically formed by a longer and a shorter single-stranded nucleic acid or by two single stranded nucleic acids, which are about equal in length, wherein one single-stranded nucleic acid is in part complementary to the other single-stranded nucleic acid and both thus form a double-stranded nucleic acid in this region, i.e.

a partially double-stranded or partially single stranded nucleic acid). Even more preferably, the at least one "complexed precomplexed nucleic acid" may be a RNA, more preferably an mRNA, even more preferably a (linear) single stranded mRNA.

According to another embodiment, the nucleic acid (molecule) used for preparation of the at least one "complexed precomplexed nucleic acid" according to the present invention comprises a length of about 5 to about 20000 nucleotides, or 100 to about 20000 nucleotides, preferably of about 250 to about 20000 nucleotides, more preferably of about 500 to about 10000 nucleotides, even more preferably of about 500 to about 5000 nucleotides.

According to one embodiment defined above, the nucleic acid (molecule) used for preparation of the at least one "complexed precomplexed nucleic acid" according to the present invention may be a coding nucleic acid, preferably any nucleic acid as defined above, more preferably a coding RNA. Even more preferably, such a coding nucleic acid, particularly such a coding RNA may be a single- or a double-stranded RNA, a single-stranded RNA, and/or a circular or linear RNA, and most preferably a linear RNA, such as a (linear) single-stranded RNA, or a ((linear) single-stranded) messenger RNA (mRNA). The coding nucleic acid, particularly the coding RNA or mRNA, used for preparation of the at least one "complexed precomplexed nucleic acid" according to the present invention may further encode a protein or a peptide, which may be selected, without being restricted thereto, e.g. from therapeutically active proteins or peptides, from antigens, e.g. tumor antigens, pathogenic antigens (e.g. selected from pathogenic proteins as defined above or from animal antigens, viral antigens, protozoal antigens, bacterial antigens, allergic antigens), autoimmune antigens, or further antigens, from allergens, from antibodies, from immunostimulatory proteins or peptides, from antigen-specific T-cell receptors, or from any other proteins or peptides suitable for a specific (therapeutic) application, wherein the coding nucleic acid, particularly the coding RNA or mRNA, used for the preparation of the at least one "complexed precomplexed nucleic acid" according to the present invention may be transported into a cell, a tissue or an organism and the protein may be expressed subsequently in this cell, tissue or organism.

a) Therapeutically active proteins

In the context of the present invention, therapeutically active proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may be selected from any naturally occurring recombinant or isolated protein known to a skilled person from the prior art, particularly from any naturally occurring recombinant or isolated protein known to exhibit a therpeutic effect. Without being restricted thereto therapeutically active proteins may comprise proteins, capable of stimulating or inhibiting the signal transduction in the cell, e.g. cytokines, antibodies, etc. Therapeutically active proteins may thus comprise cytokines of class I of the family of cytokines, having 4 positionally conserved cysteine residues (CCCC) and comprising a conserved sequence motif Trp-Ser-X-Trp-Ser (WSXWS), wherein X is a non-conserved amino acid. Cytokines of class I of the family of cytokines comprise the GM-CSF subfamily, e.g. IL-3, IL-5, GM-CSF, the IL-6-subfamily, e.g. IL-6, IL-11, IL-12, or the IL-2-subfamily, e.g. IL-2, IL-4, IL-7, IL-9, IL-15, etc., or the cytokines IL-1alpha, IL-1beta, IL-10 etc. Therapeutically active proteins may also comprise cytokines of class II of the family of cytokines, which also comprise 4 positionally conserved cystein residues (CCCC), but no conserved sequence motif Trp-Ser-X-Trp-Ser (WSXWS). Cytokines of class II of the family of cytokines comprise e.g. IFN-alpha, IFN-beta, IFN-gamma, etc. Therapeutically active proteins may additionally comprise cytokines of the family of tumor necrose factors, e.g. TNF-alpha, TNF-beta, etc., or cytokines of the family of chemokines, which comprise 7 transmembrane helices and interact with G-protein, e.g. IL-8, MIP-1, RANTES, CCR5, CXR4, etc., or cytokine specific receptors, such as TNF-RI, TNF-RII, CD40, OX40 (CD134), Fas, etc.

Therapeutically active proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may furthermore be selected from proteins associated with genetic diseases as mentioned herein, particularly proteins, which are not or not sufficiently expressed in the context of these genetic diseases.

Therapeutically active proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present

invention, may also be selected from any of the proteins given in the following: 0ATL3, 0FC3, 0PA3, 0PD2, 4-1BBL, 5T4, 6Ckine, 707-AP, 9D7, A2M, AA, AAAS, AACT, AASS, ABAT, ABCA1, ABCA4, ABCB1, ABCB11, ABCB2, ABCB4, ABCB7, ABCC2, ABCC6, ABCC8, ABCD1, ABCD3, ABCG5, ABCG8, ABL1, ABO, ABR ACAA1, ACACA, ACADL, ACADM, ACADS, ACADVL, ACAT1, ACCPN, ACE, ACHE, ACHM3, ACHM1, ACLS, ACPI, ACTA1, ACTC, ACTN4, ACVRL1, AD2, ADA, ADAMTS13, ADAMTS2, ADFN, ADH1B, ADH1C, ADLDH3A2, ADRB2, ADRB3, ADSL, AEZ, AFA, AFD1, AFP, AGA, AGL, AGMX2, AGPS, AGS1, AGT, AGTR1, AGXT, AH02, AHCY, AHDS, AHHR, AHSG, AIC, AIED, AIH2, AIH3, AIM-2, AIPL1, AIRE, AK1, ALAD, ALAS2, ALB, HPG1, ALDH2, ALDH3A2, ALDH4A1, ALDH5A1, ALDH1A1, ALDOA, ALDOB, ALMS1, ALPL, ALPP, ALS2, ALX4, AMACR, AMBP, AMCD, AMCD1, AMCN, AMELX, AMELY, AMGL, AMH, AMHR2, AMPD3, AMPD1, AMT, ANC, ANCR, ANK1, ANOP1, AOM, AP0A4, AP0C2, AP0C3, AP3B1, APC, aPKC, APOA2, APOA1, APOB, APOC3, APOC2, APOE, APOH, APP, APRT, APS1, AQP2, AR, ARAF1, ARG1, ARHGEF12, ARMET, ARSA, ARSB, ARSC2, ARSE, ART-4, ARTC1/m, ARTS, ARVD1, ARX, AS, ASAH, ASAT, ASD1, ASL, ASMD, ASMT, ASNS, ASPA, ASS, ASSP2, ASSP5, ASSP6, AT3, ATD, ATHS, ATM, ATP2A1, ATP2A2, ATP2C1, ATP6B1, ATP7A, ATP7B, ATP8B1, ATPSK2, ATRX, ATXN1, ATXN2, ATXN3, AUTS1, AVMD, AVP, AVPR2, AVSD1, AXIN1, AXIN2, AZF2, B2M, B4GALT7, B7H4, BAGE, BAGE-1, BAX, BBS2, BBS3, BBS4, BCA225, BCAA, BCH, BCHE, BCKDHA, BCKDHB, BCL10, BCL2, BCL3, BCL5, BCL6, BCPM, BCR, BCR/ABL, BDC, BDE, BDMF, BDMR, BEST1, beta-Catenin/m, BF, BFHD, BFIC, BFLS, BFSP2, BGLAP,BGN, BHD, BHR1, BING-4, BIRC5, BJS, BLM, BLMH, BLNK, BMPR2, BPGM, BRAF, BRCA1, BRCA1/m, BRCA2, BRCA2/m, BRCD2, BRCD1, BRDT, BSCL, BSCL2, BTAA, BTD, BTK, BUB1, BWS, BZX, C0L2A1, C0L6A1, C1NH, C1QA, C1QB, C1QG, C1S, C2, C3, C4A, C4B, C5, C6, C7, C7orf2, C8A, C8B, C9, CA125, CA15-3/CA 27-29, CA195, CA19-9, CA72-4, CA2, CA242, CA50, CABYR, CACD, CACNA2D1, CACNA1A, CACNA1F, CACNA1S, CACNB2, CACNB4, CAGE, CA1, CALB3, CALCA, CALCR, CALM, CALR, CAM43, CAMEL, CAP-1, CAPN3, CARD15, CASP-5/m, CASP-8, CASP-8/m, CASR, CAT, CATM, CAV3, CB1, CBBM, CBS, CCA1, CCAL2, CCAL1, CCAT, CCL-1, CCL-11, CCL-12, CCL-13, CCL-14, CCL-15, CCL-16, CCL-17, CCL-18, CCL-19, CCL-2, CCL-20, CCL-21, CCL-22, CCL-23, CCL-24, CCL-25, CCL-27, CCL-3, CCL-4, CCL-5, CCL-7, CCL-8, CCM1, CCNB1, CCND1, CCO, CCR2, CCR5, CCT, CCV, CCZS, CD1, CD19, CD20, CD22, CD25, CD27, CD27L, cD3, CD30, CD30, CD30L, CD33, CD36, CD3E, CD3G,

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PMM2, PMP22, PMS2, PMS1, PNKD, PNLIP, POF1, POLA, POLH, POMC, PON2, PON1, PORC, POTE, POU1F1, POU3F4, POU4F3, POU1F1, PPAC, PPARG, PPCD, PPGB, PPH1, PPKB, PPMX, PPOX, PPP1R3A, PPP2R2B, PPT1, PRAME, PRB, PRB3, PRCA1, PRCC, PRD, PRDX5/m, PRF1, PRG4, PRKAR1A, PRKCA, PRKDC, PRKWNK4, PRNP, PROC, PRODH, PROM1, PROP1, PROS1, PRST, PRP8, PRPF31, PRPF8, PRPH2, PRPS2, PRPS1, PRS, PRSS7, PRSS1, PRTN3, PRX, PSA, PSAP, PSCA, PSEN2, PSEN1, PSG1, PSGR, PSM, PSMA, PSORS1, PTC, PTCH, PTCH1, PTCH2, PTEN, PTGS1, PTH, PTHR1, PTLAH, PTOS1, PTPN12, PTPNI l, PTPRK, PTPRK/m, PTS, PUJO, PVR, PVRL1, PWCR, PXE, PXMP3, PXR1, PYGL, PYGM, QDPR, RAB27A, RAD54B, RAD54L, RAG2, RAGE, RAGE-1, RAG1, RAP1, RARA, RASA1, RBAF600/m, RB1, RBP4, RBP4, RBS, RCA1, RCAS1, RCCP2, RCD1, RCV1, RDH5, RDPA, RDS, RECQL2, RECQL3, RECQL4, REG1A, REHOBE, REN, RENBP, RENS1, RET, RFX5, RFXANK, RFXAP, RGR, RHAG, RHAMM/CD168, RHD, RHO, Rip-1, RLBP1, RLN2, RLN1, RLS, RMD1, RMRP, ROM1, ROR2, RP, RP1, RP14, RP17, RP2, RP6, RP9, RPD1, RPE65, RPGR, RPGRIP1, RP1, RP10, RPS19, RPS2, RPS4X, RPS4Y, RPS6KA3, RRAS2, RS1, RSN, RSS, RU1, RU2, RUNX2,RUNXl, RWS, RYR1, S-100, SAA1, SACS, SAG, SAGE, SALL1, SARDH, SART1, SART2 , SART3, SAS, SAX1, SCA2, SCA4, SCA5, SCA7, SCA8, SCA1, SCC, SCCD, SCF, SCLC1, SCN1A, SCN1B, SCN4A, SCN5A, SCNN1A, SCNN1B, SCNN1G, SCO2, SCP1, SCZD2, SCZD3, SCZD4, SCZD6, SCZD1, SDF-1 / SDHA, SDHD, SDYS, SEDL, SERPENA7, SERPINA3, SERPINA6, SERPINA1, SERPINC1, SERPIND1, SERPINE1, SERPINF2, SERPING1, SERPINI1, SFTPA1, SFTPB, SFTPC, SFTPD, SGCA, SGCB, SGCD, SGCE, SGM1, SGSH, SGY-1, SH2D1A, SHBG, SHFM2, SHFM3, SHFM1, SHH, SHOX, SI, SIAL, SIALYL LEWISX , SIASD, S11, SIM1, SIRT2/m, SIX3, SJS1, SKP2, SLC10A2, SLC12A1, SLC12A3, SLC17A5, SLC19A2, SLC22A1L, SLC22A5, SLC25A13, SLC25A15, SLC25A20, SLC25A4, SLC25A5, SLC25A6, SLC26A2, SLC26A3, SLC26A4, SLC2A1, SLC2A2, SLC2A4, SLC3A1, SLC4A1, SLC4A4, SLC5A1, SLC5A5, SLC6A2, SLC6A3, SLC6A4, SLC7A7, SLC7A9, SLC11A1, SLOS, SMA, SMAD1, SMAL, SMARCB1, SMAX2, SMCR, SMCY, SM1, SMN2, SMN1, SMPD1, SNCA, SNRPN, SOD2, SOD3, SOD1, SOS1, SOST, SOX9, SOX10, Sp17, SPANXC, SPG23, SPG3A, SPG4, SPG5A, SPG5B, SPG6, SPG7, SPINK1, SPINK5, SPPK, SPPM, SPSMA, SPTA1, SPTB, SPTLC1, SRC, SRD5A2, SRPX, SRS, SRY, ßhCG, SSTR2, SSX1, SSX2 (HOM-MEL-40/SSX2), SSX4, ST8, STAMP-1, STAR, STARP1, STATH, STEAP, STK2, STK11, STn/ KLH, STO, STOM, STS, SUOX, SURF1, SURVIVIN-2B, SYCP1, SYM1, SYN1, SYNS1, SYP, SYT/SSX, SYT-SSX-1,

SYT-SSX-2, TA-90, TAAL6, TACSTD1, TACSTD2, TAG72, TAF7L, TAF1, TAGE, TAG-72, TALI, TAM, TAP2, TAP1, TAPVR1, TARC, TARP, TAT, TAZ, TBP, TBX22, TBX3, TBX5, TBXA2R, TBXAS1, TCAP, TCF2, TCF1, TCIRG1, TCL2, TCL4, TCL1A, TCN2, TCOF1, TCR, TCRA, TDD, TDFA, TDRD1, TECK, TECTA, TEK, TEL/AML1, TELAB1, TEX15, TF, TFAP2B, TFE3, TFR2, TG, TGFA, TGF- β, TGFBI, TGFB1, TGFBR2, TGFBRE, TGFβ, TGFβRII, TGIF, TGM-4, TGM1, TH, THAS, THBD, THC, THC2, THM, THPO, THRA, THRB, TIMM8A, TIMP2, TIMP3, TIMP1, TITF1, TKCR, TKT, TLP, TLR1, TLR10, TLR2, TLR3, TLR4, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLX1, TM4SF1, TM4SF2, TMC1, TMD, TMIP, TNDM, TNF, TNFRSF11A, TNFRSF1A, TNFRSF6, TNFSF5, TNFSF6, TNFα, TNFβ, TNNI3, TNNT2, TOC, TOP2A, TOP1, TP53, TP63, TPA, TPBG, TPI, TPI/m, TPI1, TPM3, TPM1, TPMT, TPO, TPS, TPTA, TRA, TRAG3, TRAPPC2, TRC8, TREH, TRG, TRH, TRIM32, TRIM37, TRP1, TRP2, TRP-2/6b, TRP-2/INT2, Trp-p8, TRPS1, TS, TSC2, TSC3, TSC1, TSG101, TSHB, TSHR, TSP-180, TST, TTGA2B, TTN, TTPA, TTR, TU M2-PK, TULP1, TWIST, TYH, TYR, TYROBP, TYROBP, TYRP1, TYS, UBE2A, UBE3A, UBE1, UCHL1, UFS, UGT1A, ULR, UMPK, UMPS, UOX, UPA, UQCRC1, URO5, UROD, UPK1B, UROS, USH2A, USH3A, USH1A, USH1C, USP9Y, UV24, VBCH, VCF, VDI, VDR, VEGF, VEGFR-2, VEGFR-1, VEGFR-2/FLK-1, VHL, VIM, VMD2, VMD1, VMGLOM, VNEZ, VNF, VP, VRNI, VWF, VWS, WAS, WBS2, WFS2, WFS1, WHCR, WHN, WISP3, WMS, WRN, WS2A, WS2B, WSN, WSS, WT2, WT3, WT1, WTS, WWS, XAGE, XDH, XIC, XIST, XK, XM, XPA, XPC, XRCC9, XS, ZAP70, ZFHX1B, ZFX, ZFY, ZIC2, ZIC3, ZNF145, ZNF261, ZNF35, ZNF41, ZNF6, ZNF198, ZWS1.

Therapeutically active proteins, which may be encoded by the the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may further be selected from apoptotic factors or apoptosis related proteins including AIF, Apaf e.g. Apaf-1, Apaf-2, Apaf-3, oder APO-2 (L), APO-3 (L), Apopain, Bad, Bak, Bax, Bcl-2, Bcl-x_L, Bcl-x_S, bik, CAD, Calpain, Caspase e.g. Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, ced-3, ced-9, c-Jun, c-Myc, crm A, cytochrom C, CdR1, DcR1, DD, DED, DISC, DNA-PK_{CS}, DR3, DR4, DR5, FADD/MORT-1, FAK, Fas (Fas-ligand CD95/fas (receptor)), FLICE/MACH, FLIP, fodrin, fos, G-Actin, Gas-2, gelsolin, granzyme A/B, ICAD, ICE, JNK, lamin A/B, MAP, MCL-1, Mdm-2, MEKK-1, MORT-1, NEDD, $NF\text{-}_{kappa}B$, NuMa, p53, PAK-2, PARP, perforin, PITSLRE, PKCdelta, pRb,

presenilin, prICE, RAIDD, Ras, RIP, sphingomyelinase, thymidinkinase from herpes simplex, TRADD, TRAF2, TRAIL-R1, TRAIL-R2, TRAIL-R3, transglutaminase, etc.

A therapeutically active protein, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, can also be an adjuvant protein. In this context, an adjuvant protein is preferably to be understood as any protein, which is capable to elicit an innate immune response as defined herein. Preferably, such an innate immune response comprises activation of a pattern recognition receptor, such as e.g. a receptor selected from the Toll-like receptor (TLR) familiy, including e.g. a Toll like receptor selected from human TLR1 to TLR10 or from murine Toll like receptors TLR1 to TLR13. Preferably, an innate immune response is elicited in a mammal as defined above. More preferably, the adjuvant protein is selected from human adjuvant proteins or from pathogenic adjuvant proteins, in particular from bacterial adjuvant proteins. In addition, mRNA encoding huma proteins involved in adjuvant effects may be used as well.

Human adjuvant proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, typically comprise any huma protein, which is capable of eliciting an innate immune response (in a mammal), e.g. as a reaction of the binding of an exogenous TLR ligand to a TLR. More preferably, human adjuvants, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, are selected from the group consisting of, without being limited thereto, cytokines which induce or enhance an innate immune response, including IL-2, IL-12, IL-15, IL-18, IL-21CCL21, GM-CSF and TNF-alpha; cytokines which are released from macrophages, including IL-1, IL-6, IL-8, IL-12 and TNF-alpha; from components of the complement system including C1q, MBL, C1r, C1s, C2b, Bb, D, MASP-1, MASP-2, C4b, C3b, C5a, C3a, C4a, C5b, C6, C7, C8, C9, CR1, CR2, CR3, CR4, C1qR, C1INH, C4bp, MCP, DAF, H, I, P and CD59; from proteins which are components of the signalling networks of the pattern recognition receptors including TLR and IL-1R1, whereas the components are ligands of the pattern recognition receptors including IL-1alpha, IL-1 beta, Beta-defensin, heat shock proteins, such as HSP10, HSP60, HSP65, HSP70, HSP75 and HSP90, gp96, Fibrinogen, TypIII repeat extra domain A of

fibronectin; the receptors, including IL-1RI, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11; the signal transducers including components of the Small-GTPases signalling (RhoA, Ras, Rac1, Cdc42 etc.), components of the PIP signalling (PI3K, Src-Kinases, etc.), components of the MyD88-dependent signalling (MyD88, IRAK1, IRAK2, etc.), components of the MyD88-independent signalling (TICAM1, TICAM2 etc.); activated transcription factors including e.g. NF-κB, c-Fos, c-Jun, c-Myc; and induced target genes including e.g. IL-1 alpha, IL-1 beta, Beta-Defensin, IL-6, IFN gamma, IFN alpha and IFN beta; from costimulatory molecules, including CD28 or CD40-ligand or PD1; protein domains, including LAMP; cell surface proteins; or human adjuvant proteins including CD80, CD81, CD86, trif, flt-3 ligand, thymopentin, Gp96 or fibronectin, etc., or any species homolog of any of the above human adjuvant proteins.

Pathogenic adjuvant proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, typically comprise any pathogenic (adjuvant) protein, which is capable of eliciting an innate immune response (in a mammal), more preferably selected from pathogenic (adjuvant) proteins derived from bacteria, protozoa, viruses, or fungi, animals, etc., and even more preferably from pathogenic adjuvant proteins selected from the group consisting of, without being limited thereto, bacterial proteins, protozoa proteins (e.g. profilin – like protein of Toxoplasma gondii), viral proteins, or fungal proteins, animal proteins, etc.

In this context, bacterial (adjuvant) proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may comprise any bacterial protein, which is capable of eliciting an innate immune response (preferably in a mammal) or shows an adjuvant character. More preferably, such bacterial (adjuvant) proteins are selected from the group consisting of bacterial heat shock proteins or chaperons, including Hsp60, Hsp70, Hsp90, Hsp100; OmpA (Outer membrane protein) from gram-negative bacteria; bacterial porins, including OmpF; bacterial toxins, including pertussis toxin (PT) from *Bordetella pertussis*, pertussis adenylate cyclase toxin CyaA and CyaC from *Bordetella pertussis*, PT-9K/129G mutant from pertussis toxin, pertussis adenylate cyclase toxin CyaA and CyaC from *Bordetella pertussis*, tetanus toxin, cholera toxin (CT), cholera toxin B-subunit, CTK63

mutant from cholera toxin, CTE112K mutant from CT, *Escherichia coli* heat-labile enterotoxin (LT), B subunit from heat-labile enterotoxin (LTB) *Escherichia coli* heat-labile enterotoxin mutants with reduced toxicity, including LTK63, LTR72; phenol-soluble modulin; neutrophil-activating protein (HP-NAP) from *Helicobacter pylori*; Surfactant protein D; Outer surface protein A lipoprotein from *Borrelia burgdorferi*, Ag38 (38 kDa antigen) from *Mycobacterium tuberculosis*; proteins from bacterial fimbriae; Enterotoxin CT of Vibrio cholerae, Pilin from pili from gram negative bacteria, and Surfactant protein A; etc., or any species homolog of any of the above bacterial (adjuvant) proteins.

Bacterial (adjuvant) proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may also be selected from bacterial adjuvant proteins, even more preferably selected from the group consisting of, without being limited thereto, bacterial flagellins, including flagellins from organisms including *Agrobacterium, Aquifex, Azospirillum, Bacillus, Bartonella, Bordetella, Borrelia, Burkholderia, Campylobacter, Caulobacte, Clostridium, Escherichia, Helicobacter, Lachnospiraceae, Legionella, Listeria, Proteus, Pseudomonas, Rhizobium, Rhodobacter, Roseburia, Salmonella, Serpulina, Serratia, Shigella, Treponema, Vibrio, Wolinella, Yersinia*, more preferably flagellins from the species, without being limited thereto, *Agrobacterium tumefaciens, Aquifex pyrophilus, Azospirillum brasilense, Bacillus subtilis, Bacillus thuringiensis, Bartonella bacilliformis, Bordetella bronchiseptica, Borrelia burgdorferi, Burkholderia cepacia, Campylobacter jejuni, Caulobacter crescentus, Clostridium botulinum strain Bennett clone 1, Escherichia coli, Helicobacter pylori, Lachnospiraceae bacterium, Legionella pneumophila, Listeria monocytogenes, Proteus mirabilis, Pseudomonas aeroguinosa, Pseudomonas syringae, Rhizobium meliloti, Rhodobacter sphaeroides, Roseburia cecicola, Roseburis hominis, Salmonella typhimurium, Salmonella bongori, Salmonella typhi, Salmonella enteritidis, Serpulina hyodysenteriae, Serratia marcescens, Shigella boydii, Treponema phagedenis, Vibrio alginolyticus, Vibrio cholerae, Vibrio parahaemolyticus, Wolinella succinogenes* and *Yersinia enterocolitica*.

Bacterial flagellins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, even

more preferably comprise a sequence selected from the group comprising any of the following sequences as referred to their accession numbers:

organism	species	gene name	accession No	GI No
Agrobacterium	*Agrobacterium tumefaciens*	FlaD (flaD) FlhB (flhB) FliG (fliG) FliN (fliN) FliM (fliM) MotA (motA) FlgF (flgF) FliI (fliI) FlgB (flgB) FlgC (flgC) FliE (fliE) FlgG (flgG) FlgA (flgA) FlgI (flgI) FlgH (flgH) FliL (fliL) FliP (fliP) FlaA (flaA) FlaB (flaB) FlaC (flaC)	U95165	GI:14278870
Aquifex	*Aquifex pyrophilus*		U17575	GI:596244
Azospirillum	*Azospirillum brasilense*	Laf1	U26679	GI:1173509
Bacillus	*Bacillus subtilis*	hag	AB033501	GI:14278870
Bacillus	*Bacillus thuringiensis*	flab	X67138	GI:46019718
Bartonella	*Bartonella bacilliformis*		L20677	GI:304184
Bordetella	*Bordetella bronchiseptica*	flaA	L13034	GI:289453
Borrelia	*Borrelia burgdorferi*		X16833	GI:39356
Burkholderia	*Burkholderia cepacia*	fliC	AF011370	GI:2935154
Campylobacter	*Campylobacter jejuni*	flaA flaB	J05635	GI:144197
Caulobacter	*Caulobacter crescentus*		J01556	GI:144239
Clostridium	*Clostridium botulinum strain Bennett clone 1*	FlaA	DQ845000	GI:114054886
Escherichia	*Escherichia coli*	hag	M14358	GI:146311

			AJ 884569 (EMBL-SVA)	
Helicobacter	*Helicobacter pylori*	flaA	X60746	GI:43631
Lachnospiraceae	*Lachnospiraceae bacterium*		DQ789131	GI:113911615
Legionella	*Legionella pneumophila*	flaA	X83232	GI:602877
Listeria	*Listeria monocytogenes*	flaA	X65624	GI:44097
Proteus	*Proteus mirabilis*	FlaD (flaD) FlaA (flaA) FlaB (flaB) FliA (fliA) FliZ (fliZ)	AF221596	GI:6959881
Pseudomonas	*Pseudomonas aeroguinosa*	flaA	M57501	GI:151225
Pseudomonas	*Pseudomonas syringae*	fliC	EF544882	GI:146335619
Rhizobium	*Rhizobium meliloti*	flaA flaB	M24526	GI:152220
Rhodobacter	*Rhodobacter sphaeroides*	fliC	AF274346	GI:10716972
Roseburia	*Roseburia cecicola*		M20983	GI:152535
Roseburia	*Roseburis hominis*	Fla2	DQ789141	GI:113911632
Salmonella	*Salmonella typhimurium*		D13689 (NCBI ID)	GI:217062
Salmonella	*Salmonella bongori*	fliC	AY603412	GI:51342390
Salmonella	*Salmonella typhi*	flag	L21912	GI:397810
Salmonella	*Salmonella enteritidis*	fliC	M84980	GI:154015
Serpulina	*Serpulina hyodysenteriae*	flaB2	X63513	GI:450669
Serratia	*Serratia marcescens*	hag	M27219	GI:152826
Shigella	*Shigella boydii*	fliC-SB	D26165	GI:442485
Treponema	*Treponema phagedenis*	flaB2	M94015	GI:155060
Vibrio	*Vibrio alginolyticus*	flaA	EF125175	GI:119434395
Vibrio s	*Vibrio parahaemolyticus*		AF069392	GI:7327274
Wolinella	*Wolinella succinogenes*	flag	M82917	GI:155337
Yersinia	*Yersinia*		L33467	GI:496295

	enterocolitica			

Protozoa proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may be selected from any protozoa protein showing adjuvant character, more preferably, from the group consisting of, without being limited thereto, Tc52 from *Trypanosoma cruzi*, PFTG from *Trypanosoma gondii*, Protozoan heat shock proteins, LelF from *Leishmania spp.*, profilin–like protein from *Toxoplasma gondii*, etc.

Viral proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may be selected from any viral protein showing adjuvant character, more preferably, from the group consisting of, without being limited thereto, Respiratory Syncytial Virus fusion glycoprotein (F-protein), envelope protein from MMT virus, mouse leukemia virus protein, Hemagglutinin protein of wild-type measles virus, etc.

Fungal proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may be selected from any fungal protein showing adjuvant character, more preferably, from the group consisting of, without being limited thereto, fungal immunomodulatory protein (FIP; LZ-8), etc.

Finally, pathogenic adjuvant proteins, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may be selected from any further pathogenic protein showing adjuvant character, more preferably, from the group consisting of, without being limited thereto, Keyhole limpet hemocyanin (KLH), OspA, etc.

b) Antigens

The nucleic acid (molecule), preferably an mRNA, of the at least one "complexed precomplexed nucleic acid" according to the present invention, may alternatively encode an antigen. According to the present invention, the term "antigen" refers to a substance which is recognized by the immune system and is capable of triggering an antigen-

specific immune response, e.g. by formation of antibodies as part of an adaptive immune response. In this context, the first step of an adaptive immune response is the activation of native antigen-specific T cells by antigen-presenting cells. This occurs in the lymphoid tissues and organs through which native T cells are constantly passing. The three cell types that can serve as antigen-presenting cells are dendritic cells, macrophages, and B cells. Each of these cells has a distinct function in eliciting immune responses. Tissue dendritic cells take up antigens by phagocytosis and macropinocytosis and are stimulated by infection to migrate to the local lymphoid tissue, where they differentiate into mature dendritic cells. Macrophages ingest particulate antigens such as bacteria and are induced by infectious agents to express MHC class II molecules. The unique ability of B cells to bind and internalize soluble protein antigens via their receptors may be important to induce T cells. Presenting the antigen on MHC molecules leads to activation of T cells which induces their proliferation and differentiation into armed effector T cells. The most important function of effector T cells is the killing of infected cells by $CD8^+$ cytotoxic T cells and the activation of macrophages by TH1 cells which together make up cell-mediated immunity, and the activation of B cells by both TH2 and TH1 cells to produce different classes of antibody, thus driving the humoral immune response. T cells recognize an antigen by their T cell receptors which does not recognize and bind antigen directly, but instead recognize short peptide fragments e.g. of pathogens' protein antigens, which are bound to MHC molecules on the surfaces of other cells.

T cells fall into two major classes that have different effector functions. The two classes are distinguished by the expression of the cell-surface proteins CD4 and CD8. These two types of T cells differ in the class of MHC molecule that they recognize. There are two classes of MHC molecule- MHC class I and MHC class II- which differ in their structure and expression pattern on tissues of the body. $CD4^+$ T cells bind to the MHC class II molecule and $CD8^+$ T cells to the MHC class I molecule. MHC class I and MHC class II have distinct distributions among cells that reflect the different effector functions of the T cells that recognize them. MHC class I molecules present peptides from pathogens, commonly viruses to $CD8^+$ T cells, which differentiate into cytotoxic T cells that are specialized to kill any cell that they specifically recognize. Almost all cells express MHC class I molecules, although the level of constitutive expression varies from one cell type to the next. But not only pathogenic peptides from viruses are presented by MHC class I

molecules, also self-antigens like tumour antigens are presented by them. MHC class I molecules bind peptides from proteins degraded in the cytosol and transported in the endoplasmic reticulum. Thereby MHC class I molecules on the surface of cells infected with viruses or other cytosolic pathogens display peptides from these pathogen. The $CD8^+$ T cells that recognize MHC class I:peptide complexes are specialized to kill any cells displaying foreign peptides and so rid the body of cells infected with viruses and other cytosolic pathogens. The main function of $CD4^+$ T cells ($CD4^+$ helper T cells) that recognize MHC class II molecules is to activate other effector cells of the immune system. Thus MHC class II molecules are normally found on B lymphocytes, dendritic cells, and macrophages, cells that participate in immune responses, but not on other tissue cells. Macrophages, for example, are activated to kill the intravesicular pathogens they harbour, and B cells to secrete immunoglobulins against foreign molecules. MHC class II molecules are prevented from binding to peptides in the endoplasmic reticulum and thus MHC class II molecules bind peptides from proteins which are degraded in endosomes. They can capture peptides from pathogens that have entered the vesicular system of macrophages, or from antigens internalized by immature dendritic cells or the immunoglobulin receptors of B cells. Pathogens that accumulate in large numbers inside macrophage and dendritic cell vesicles tend to stimulate the differentiation of TH1 cells, whereas extracellular antigens tend to stimulate the production of TH2 cells. TH1 cells activate the microbicidal properties of macrophages and induce B cells to make IgG antibodies that are very effective of opsonising extracellular pathogens for ingestion by phagocytic cells, whereas TH2 cells initiate the humoral response by activating native B cells to secrete IgM, and induce the production of weakly opsonising antibodes such as IgG1 and IgG3 (mouse) and IgG2 and IgG4 (human) as well as IgA and IgE (mouse and human).

In the context of the present invention, antigens, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, typically comprise any antigen, falling under the above definition, more preferably protein and peptide antigens, e.g. antigen, including tumor antigens, pathogenic antigens or pathogens, animal antigens, viral antigens, protozoal antigens, bacterial antigens, allergy antigens, or autoimmune (self-)antigens, etc. In accordance with the invention, antigens, which may be encoded by the nucleic acid (molecule) of

the at least one "complexed precomplexed nucleic acid" according to the present invention, may be antigens generated outside the cell, more typically antigens not derived from the host organism (e.g. a human) itself (i.e. non-self antigens) but rather derived from host cells outside the host organism, e.g. viral antigens, bacterial antigens, fungal antigens, protozoological antigens, animal antigens (preferably selected from animals or organisms as disclosed herein), allergy antigens, etc. Allergy antigens are typically antigens, which cause an allergy in a human and may be derived from either a human or other sources. Antigens, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may be furthermore antigens generated inside the cell, the tissue or the body, e.g. by secretion of proteins, their degradation, metabolism, etc. Such antigens include antigens derived from the host organism (e.g. a human) itself, e.g. tumor antigens, self-antigens or auto-antigens, such as auto-immune self-antigens, etc., but also (non-self) antigens as defined above, which have been originally been derived from host cells outside the host organism, but which are fragmented or degraded inside the body, tissue or cell, e.g. by (protease) degradation, metabolism, etc.

Antigens, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may furthermore comprise fragments or variants of such antigens as mentioned herein, particularly of protein or peptide antigens. Fragments of such antigens in the context of the present invention may comprise fragments preferably having a length of about 6 to about 20 or even more amino acids, e.g. fragments as processed and presented by MHC class I molecules, preferably having a length of about 8 to about 10 amino acids, e.g. 8, 9, or 10, (or even 11, or 12 amino acids), or fragments as processed and presented by MHC class II molecules, preferably having a length of about 13 or more amino acids, e.g. 13, 14, 15, 16, 17, 18, 19, 20 or even more amino acids, wherein these fragments may be selected from any part of the amino acid sequence. These fragments are typically recognized by T-cells in form of a complex consisting of the peptide fragment and an MHC molecule, i.e. the fragments are typically not recognized in their native form.

Fragments of antigens as defined herein may also comprise epitopes of those antigens. Epitopes (also called "antigen determinants") are typically fragments located on the outer

surface of (native) protein or peptide antigens as defined herein, preferably having 5 to 15 amino acids, more preferably having 5 to 12 amino acids, even more preferably having 6 to 9 amino acids, which may be recognized by antibodies, i.e. in their native form.

One class of antigens, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, comprises tumor antigens. "Tumor antigens" are preferably located on the surface of the (tumor) cell. Tumor antigens may also be selected from proteins, which are overexpressed in tumor cells compared to a normal cell. Furthermore, tumor antigens also includes antigens expressed in cells which are (were) not themselves (or originally not themselves) degenerate but are associated with the supposed tumor. Antigens which are connected with tumor-supplying vessels or (re)formation thereof, in particular those antigens which are associated with neovascularization, e.g. growth factors, such as VEGF, bFGF etc., are also included herein. Antigens connected with a tumor furthermore include antigens from cells or tissues, typically embedding the tumor. Further, some substances (usually proteins or peptides) are expressed in patients suffering (knowingly or not-knowingly) from a cancer disease and they occur in increased concentrations in the body fluids of said patients. These substances are also referred to as "tumor antigens", however they are not antigens in the stringent meaning of an immune response inducing substance. The class of tumor antigens can be divided further into tumor-specific antigens (TSAs) and tumor-associated-antigens (TAAs). TSAs can only be presented by tumor cells and never by normal "healthy" cells. They typically result from a tumor specific mutation. TAAs, which are more common, are usually presented by both tumor and healthy cells. These antigens are recognized and the antigen-presenting cell can be destroyed by cytotoxic T cells. Additionally, tumor antigens can also occur on the surface of the tumor in the form of, e.g., a mutated receptor. In this case, they can be recognized by antibodies.

Examples of tumor antigens, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, are shown in Tables 1 and 2 below. These tables illustrate specific (protein) antigens (i.e. "tumor antigens") with respect to the cancer disease, they are associated with. According

to the invention, the terms "cancer diseases" and "tumor diseases" are used synonymously herein.

Table 1: Antigens expressed in cancer diseases

Tumor antigen	*Name of tumor antigen*	*Cancers or cancer diseases related thereto*
5T4		colorectal cancer, gastric cancer, ovarian cancer
707-AP	707 alanine proline	Melanoma
9D7		renal cell carcinoma
AFP	alpha-fetoprotein	hepatocellular carcinoma, gallbladder cancer, testicular cancer, ovarian cancer, bladder cancer
AlbZIP HPG1		prostate cancer
alpha5beta1-Integrin		
alpha5beta6-Integrin		colon cancer
alpha-methylacyl-coenzyme A racemase		prostate cancer
ART-4	adenocarcinoma antigen recognized by T cells 4	lung cancer, head and neck cancer, leukemia, esophageal cancer, gastric cancer, cervical cancer, ovarian cancer, breast cancer, squamous cell carcinoma
B7H4		ovarian cancer
BAGE-1	B antigen	bladder cancer, head and neck cancer, lung cancer, melanoma, squamous cell carcinoma
BCL-2		leukemia
BING-4		melanoma
CA 15-3/CA 27-29		breast cancer, ovary cancer, lung cancer, prostate cancer
CA 19-9		gastric cancer, pancreatic cancer, liver cancer, breast cancer, gallbladder cancer, colon cancer, ovary cancer, lung cancer
CA 72-4		ovarian cancer
CA125		ovarian cancer, colorectal cancer, gastric cancer, liver cancer, pancreatic cancer, uterus cancer, cervix carcinoma, colon cancer, breast cancer, lung cancer
calreticulin		bladder cancer
CAMEL	CTL-recognized antigen on melanoma	melanoma
CASP-8	caspase-8	head and neck cancer
cathepsin B		breast cancer
cathepsin L		breast cancer

CD19		B-cell malignancies
CD20		
CD22		
CD25		
CD30		
CD33		
CD4		
CD52		
CD55		
CD56		
CD80		
CEA	carcinoembryonic antigen	gut carcinoma, colorectal cancer, colon cancer, hepatocellular cancer, lung cancer, breast cancer, thyroid cancer, pancreatic cancer, liver cancer cervix cancer, bladder cancer, melanoma
CLCA2	calcium-activated chloride channel-2	lung cancer
CML28		leukemia
Coactosin-like protein		pancreatic cancer
Collagen XXIII		prostate cancer
COX-2		ovarian cancer, breast cancer, colorectal cancer
CT-9/BRD6	bromodomain testis-specific protein	
Cten	C-terminal tensin-like protein	prostate cancer
cyclin B1		
cyclin D1		ovarian cancer
cyp-B	cyclophilin B	bladder cancer, lung cancer, T-cell leukemia, squamous cell carcinoma,
CYPB1	cytochrom P450 1B1	leukemia
DAM-10/MAGE-B1	differentiation antigen melanoma 10	melanoma, skin tumors, ovarian cancer, lung cancer
DAM-6/MAGE-B2	differentiation antigen melanoma 6	melanoma, skin tumors, ovarian cancer, lung cancer
EGFR/Her1		lung cancer, ovarian cancer, head and neck cancer, colon cancer, pancreatic cancer, breast cancer
EMMPRIN	tumor cell-associated extracellular matrix metalloproteinase inducer/	lung cancer, breast cancer, bladder cancer, ovarian cancer, brain cancer, lymphoma
EpCam	epithelial cell adhesion molecule	ovarian cancer, breast cancer, colon cancer, lung cancer
EphA2	ephrin type-A receptor 2	glioma
EphA3	ephrin type-A receptor 2	melanoma, sarcoma, lung cancer
ErbB3		breast cancer
EZH2	(enhancer of Zeste homolog 2)	endometrium cancer, melanoma, prostate cancer, breast cancer
FGF-5	fibroblast growth factor-5	renal cell carcinoma, breast cancer, prostate cancer
FN	fibronectin	melanoma

Fra-1	Fos-related antigen-1	breast cancer, esophageal cancer, renal cell carcinoma, thyroid cancer
G250/CAIX	glycoprotein 250	leukemia, renal cell carcinoma, head and neck cancer, colon cancer, ovarian cancer, cervical cancer
GAGE-1	G antigen 1	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-2	G antigen 2	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-3	G antigen 3	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-4	G antigen 4	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-5	G antigen 5	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-6	G antigen 6	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-7b	G antigen 7b	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-8	G antigen 8	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GDEP	gene differentially expressed in prostate	prostate cancer
GnT-V	N-acetylglucosaminyltransferase V	glioma, melanoma
gp100	glycoprotein 100 kDa	melanoma
GPC3	glypican 3	hepatocellular carcinoma, melanoma
HAGE	helicase antigen	bladder cancer
HAST-2	human signet ring tumor-2	
hepsin		prostate
Her2/neu/ErbB2	human epidermal receptor-2/neurological	breast cancer, bladder cancer, melanoma, ovarian cancer, pancreas cancer, gastric cancer
HERV-K-MEL		melanoma
HNE	human neutrophil elastase	leukemia
homeobox NKX 3.1		prostate cancer
HOM-TES-14/SCP-1		ovarian cancer
HOM-TES-85		
HPV-E6		cervical cancer
HPV-E7		cervical cancer
HST-2		gastric cancer
hTERT	human telomerase reverse transcriptase	breast cancer, melanoma, lung cancer, ovarian cancer, sarcoma,

		Non-Hodgkin-lymphoma, acute leukemia
iCE	intestinal carboxyl esterase	renal cell carcinoma
IGF-1R		colorectal cancer
IL-13Ra2	interleukin 13 receptor alpha 2 chain	glioblastoma
IL-2R		colorectal cancer
IL-5		
immature laminin receptor		renal cell carcinoma
kallikrein 2		prostate cancer
kallikrein 4		prostate cancer
Ki67		prostate cancer, breast cancer, Non-Hodgkin-lymphoma, melanoma
KIAA0205		bladder cancer
KK-LC-1	Kita-kyushu lung cancer antigen 1	lung cancer
KM-HN-1		tongue cancer, hepatocellular carcinomas, melanoma, gastric cancer, esophageal, colon cancer, pancreatic cancer
LAGE-1	L antigen	bladder cancer, head and neck cancer, melanoma
livin		bladder cancer, melanoma
MAGE-A1	melanoma antigen-A1	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A10	melanoma antigen-A10	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A12	melanoma antigen-A12	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia, prostate cancer, myeloma, brain tumors
MAGE-A2	melanoma antigen-A2	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A3	melanoma antigen-A3	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A4	melanoma antigen-A4	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A6	melanoma antigen-A6	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A9	melanoma-antigen-A9	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-B1	melanoma-antigen-B1	melanoma
MAGE-B10	melanoma-antigen-B10	melanoma
MAGE-B16	melanoma-antigen-B16	melanoma
MAGE-B17	melanoma-antigen-B17	melanoma

MAGE-B2	melanoma-antigen-B2	melanoma
MAGE-B3	melanoma-antigen-B3	melanoma
MAGE-B4	melanoma-antigen-B4	melanoma
MAGE-B5	melanoma-antigen-B5	melanoma
MAGE-B6	melanoma-antigen-B6	melanoma
MAGE-C1	melanoma-antigen-C1	bladder cancer, melanoma
MAGE-C2	melanoma-antigen-C2	melanoma
MAGE-C3	melanoma-antigen-C3	melanoma
MAGE-D1	melanoma-antigen-D1	melanoma
MAGE-D2	melanoma-antigen-D2	melanoma
MAGE-D4	melanoma-antigen-D4	melanoma
MAGE-E1	melanoma-antigen-E1	bladder cancer, melanoma
MAGE-E2	melanoma-antigen-E2	melanoma
MAGE-F1	melanoma-antigen-F1	melanoma
MAGE-H1	melanoma-antigen-H1	melanoma
MAGEL2	MAGE-like 2	melanoma
mammaglobin A		breast cancer
MART-1/Melan-A	melanoma antigen recognized by T cells-1/melanoma antigen A	melanoma
MART-2	melanoma antigen recognized by T cells-2	melanoma
matrix protein 22		bladder cancer
MC1R	melanocortin 1 receptor	melanoma
M-CSF	macrophage colony-stimulating factor gene	ovarian cancer
mesothelin		ovarian cancer
MG50/PXDN		breast cancer, glioblastoma, melanoma
MMP 11	M-phase phosphoprotein 11	leukemia
MN/CA IX-antigen		renal cell carcinoma
MRP-3	multidrug resistance-associated protein 3	lung cancer
MUC1	mucin 1	breast cancer
MUC2	mucin 2	breast cancer, ovarian cancer, pancreatic cancer
NA88-A	NA cDNA clone of patient M88	melanoma
N-acetylglucos-aminyltransferase -V		
Neo-PAP	Neo-poly(A) polymerase	
NGEP		prostate cancer
NMP22		bladder cancer
NPM/ALK	nucleophosmin/anaplastic lymphoma kinase fusion protein	
NSE	neuron-specific enolase	small cell cancer of lung, neuroblastoma, Wilm' tumor, melanoma, thyroid cancer, kidney cancer, testicle cancer, pancreas cancer
NY-ESO-1	New York esophageous 1	bladder cancer, head and neck cancer, melanoma, sarcoma, B-lymphoma, hepatoma, pancreatic

		cancer, ovarian cancer, breast cancer
NY-ESO-B		
OA1	ocular albinism type 1 protein	melanoma
OFA-iLRP	oncofetal antigen-immature laminin receptor	leukemia
OGT	O-linked N-acetylglucosamine transferase gene	
OS-9		
osteocalcin		prostate cancer
osteopontin		prostate cancer, breast cancer, ovarian cancer
p15	protein 15	
p15		melanoma
p190 minor bcr-abl		
p53		
PAGE-4	prostate GAGE-like protein-4	prostate cancer
PAI-1	plasminogen acitvator inhibitor 1	breast cancer
PAI-2	plasminogen acitvator inhibitor 2	breast cancer
PAP	prostate acic phosphatase	prostate cancer
PART-1		prostate cancer
PATE		prostate cancer
PDEF		prostate cancer
Pim-1-Kinase		
Pin1	Propyl isomerase	prostate cancer
POTE		prostate cancer
PRAME	preferentially expressed antigen of melanoma	melanoma, lung cancer, leukemia, head and neck cancer, renal cell carcinoma, sarcoma
prostein		prostate cancer
proteinase-3		
PSA	prostate-specific antigen	prostate cancer
PSCA		prostate cancer
PSGR		prostate cancer
PSM		
PSMA	prostate-specific membrane antigen	prostate cancer
RAGE-1	renal antigen	bladder cancer, renal cancer, sarcoma, colon cancer
RHAMM/CD168	receptor for hyaluronic acid mediated motility	leukemia
RU1	renal ubiquitous 1	bladder cancer, melanoma, renal cancer
RU2	renal ubiquitous 1	bladder cancer, melanoma, sarcoma, brain tumor, esophagel cancer, renal cancer, colon cancer, breast cancer
S-100		melanoma
SAGE	sarcoma antigen	
SART-1	squamous antigen rejecting tumor 1	esophageal cancer, head and neck cancer, lung cancer, uterine cancer
SART-2	squamous antigen rejecting tumor 1	head and neck cancer, lung cancer, renal cell carcinoma, melanoma,

		brain tumor
SART-3	squamous antigen rejecting tumor 1	head and neck cancer, lung cancer, leukemia, melanoma, esophageal cancer
SCC	squamous cell carcinoma antigen	lung cancer
Sp17	sperm protein 17	multiple myeloma
SSX-1	synovial sarcoma X breakpoint 1	hepatocellular cell carcinom, breast cancer
SSX-2/HOM-MEL-40	synovial sarcoma X breakpoint 2	breast cancer
SSX-4	synovial sarcoma X breakpoint 4	bladder cancer, hepatocellular cell carcinoma, breast cancer
STAMP-1		prostate cancer
STEAP	six transmembrane epithelial antigen prostate	prostate cancer
survivin		bladder cancer
survivin-2B	intron 2-retaining survivin	bladder cancer
TA-90		melanoma
TAG-72		prostate carcinoma
TARP		prostate cancer
TGFb	TGFbeta	
TGFbRII	TGFbeta receptor II	
TGM-4	prostate-specific transglutaminase	prostate cancer
TRAG-3	taxol resistant associated protein 3	breast cancer, leukemia, and melanoma
TRG	testin-related gene	
TRP-1	tyrosine related protein 1	melanoma
TRP-2/6b	TRP-2/novel exon 6b	melanoma, glioblastoma
TRP-2/INT2	TRP-2/intron 2	melanoma, glioblastoma
Trp-p8		prostate cancer
Tyrosinase		melanoma
UPA	urokinase-type plasminogen activator	breast cancer
VEGF	vascular endothelial growth factor	
VEGFR-2/FLK-1	vascular endothelial growth factor receptor-2	
WT1	Wilm' tumor gene	gastric cancer, colon cancer, lung cancer, breast cancer, ovarian cancer, leukemia

Table 2: Mutant antigens expressed in cancer diseases

Mutant antigen	*Name of mutant antigen*	*Cancers or cancer diseases related thereto*
alpha-actinin-4/m		lung carcinoma
ARTC1/m		melanoma
bcr/abl	breakpoint cluster region-Abelson fusion protein	CML
beta-Catenin/m	beta-Catenin	melanoma
BRCA1/m		breast cancer
BRCA2/m		breast cancer
CASP-5/m		colorectal cancer, gastric cancer, endometrial carcinoma

CASP-8/m		head and neck cancer, squamous cell carcinoma
CDC27/m	cell-division-cycle 27	
CDK4/m	cyclin-dependent kinase 4	melanoma
CDKN2A/m		melanoma
CML66		CML
COA-1/m		colorectal cancer
DEK-CAN	fusion protein	AML
EFTUD2/m		melanoma
ELF2/m	Elongation factor 2	lung squamous cell carcinoma
ETV6-AML1	Ets variant gene6/acute myeloid leukemia 1 gene fusion protein	ALL
FN1/m	fibronectin 1	melanoma
GPNMB/m		melanoma
HLA-A*0201-R170I	arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene	renal cell carcinoma
HLA-A11/m		melanoma
HLA-A2/m		renal cell carcinoma
HSP70-2M	heat shock protein 70-2 mutated	renal cell carcinoma, melanoma, neuroblastoma
KIAA0205/m		bladder tumor
K-Ras/m		pancreatic carcinoma, colorectal carcinoma
LDLR-FUT	LDR-Fucosyltransferase fusion protein	melanoma
MART2/m		melanoma
ME1/m		non-small cell lung carcinoma
MUM-1/m	melanoma ubiquitous mutated 1	melanoma
MUM-2/m	melanoma ubiquitous mutated 2	melanoma
MUM-3/m	melanoma ubiquitous mutated 3	melanoma
Myosin class I/m		melanoma
neo-PAP/m		melanoma
NFYC/m		lung squamous cell carcinoma
N-Ras/m		melanoma
OGT/m		colorectal carcinoma
OS-9/m		melanoma
p53/m		
Pml/RARa	promyelocytic leukemia/retinoic acid receptor alpha	APL, PML
PRDX5/m		melanoma
PTPRK/m	receptor-type protein-tyrosine phosphatase kappa	melanoma
RBAF600/m		melanoma
SIRT2/m		melanoma
SYT-SSX-1	synaptotagmin I/synovial sarcoma X fusion protein	sarcoma
SYT-SSX-2	synaptotagmin I/synovial sarcoma X fusion protein	sarcoma
TEL-AML1	translocation Ets-family leukemia/acute myeloid leukemia 1 fusion protein	AML

TGFbRII	TGFbeta receptor II	colorectal carcinoma
TPI/m	triosephosphate isomerase	Melanoma

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

STAMP-1, STEAP, survivin, survivin-2B, SYT-SSX-1, SYT-SSX-2, TA-90, TAG-72, TARP, TEL-AML1, TGFbeta, TGFbetaRII, TGM-4, TPI/m, TRAG-3, TRG, TRP-1, TRP-2/6b, TRP/INT2, TRP-p8, tyrosinase, UPA, VEGF, VEGFR-2/FLK-1, and WT1.

In a particularly preferred embodiment, the tumor antigens, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, are selected from the group consisting of MAGE-A1 (e.g. MAGE-A1 according to accession number M77481), MAGE-A2, MAGE-A3, MAGE-A6 (e.g. MAGE-A6 according to accession number NM_005363), MAGE-C1, MAGE-C2, melan-A (e.g. melan-A according to accession number NM_005511), GP100 (e.g. GP100 according to accession number M77348), tyrosinase (e.g. tyrosinase according to accession number NM_000372), survivin (e.g. survivin according to accession number AF077350), CEA (e.g. CEA according to accession number NM_004363), Her-2/neu (e.g. Her-2/neu according to accession number M11730), WT1 (e.g. WT1 according to accession number NM_000378), PRAME (e.g. PRAME according to accession number NM_006115), EGFRI (epidermal growth factor receptor 1) (e.g. EGFRI (epidermal growth factor receptor 1) according to accession number AF288738), MUC1, mucin-1 (e.g. mucin-1 according to accession number NM_002456), SEC61G (e.g. SEC61G according to accession number NM_014302), hTERT (e.g. hTERT accession number NM_198253), 5T4 (e.g. 5T4 according to accession number NM_006670), NY-Eso-1 (e.g. NY-Eso1 according to accession number NM_001327), TRP-2 (e.g. TRP-2 according to accession number NM_001922), STEAP, PCA, PSA, PSMA, etc.

One further class of antigens, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, comprises allergy antigens. Such allergy antigens may be selected from antigens derived from different sources, e.g. from animals, plants, fungi, bacteria, etc. Allergens in this context include e.g. grasses, pollens, molds, drugs, or numerous environmental triggers, etc. Allergy antigens typically belong to different classes of compounds, such as nucleic acids and their fragments, proteins or peptides and their fragments, carbohydrates, polysaccharides, sugars, lipids, phospholipids, etc. Of particular interest in the context of the present invention are antigens, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed

nucleic acid" according to the present invention, i.e. protein or peptide antigens and their fragments or epitopes, or nucleic acids and their fragments, particularly nucleic acids and their fragments, encoding such i.e. protein or peptide antigens and their fragments or epitopes.

Particularly preferred, antigens derived from animals, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may include antigens derived from, without being limited thereto, insects, such as mite (e.g. house dust mites), mosquito, bee (e.g. honey bee, bumble bee), cockroache, tick, moth (e.g. silk moth), midge, bug, flea, wasp, caterpillar, fruit fly, migratory locust, grasshopper, ant aphide, from crustaceans, such as shrimps, crab, krill, lobster, prawn, crawfish, scampi, from birds, such as duck, goose, seagull, turkey, ostrich, chicken, from fishes, such as eel, herring, carp, seabream, codfish, halibut, catfish, beluga, salmon, flounder, mackerel, cuttlefish, perch, form molluscs, such as scallop, octopus, abalone, snail, whelk, squid, clam, mussel, from spiders, from mammals, such as cow, rabbit, sheep, lion, jaguar, leopard, rat, pig, buffalo, dog, loris, hamster, guinea pig, fallow deer, horse, cat, mouse, ocelot, serval, from arthropod, such as spider, or silverfish, from worms, such as nematodes, from trichinella species, or roundworm, from amphibians, such as frogs, or from sea squirt, etc.

Antigens derived from plants, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may include antigens derived from, without being limited thereto, fruits, such as kiwi, pineapple, jackfruit,papaya, lemon, orange, mandarin, melon, sharon fruit, strawberry, lychee, apple, cherry paradise apple, mango, passion fruit, plum, apricot, nectarine, pear, passion fruit, raspberry, grape, from vegetables, such as garlic, onion, leek, soya bean, celery, cauliflower, turnip, paprika, chickpea, fennel, zucchini, cucumber, carrot, yam, bean, pea, olive, tomato, potato, lentil, lettuce, avocado, parsley, horseradish, chirimoya, beet, pumkin, spinach, from spices, such as mustard, coriander, saffron, pepper, aniseed, from crop, such as oat, buckwheat, barley, rice, wheat, maize, rapeseed, sesame, from nuts, such as cashew, walnut, butternut, pistachio, almond, hazelnut, peanut, brazil nut, pecan, chestnut, from trees, such as alder, hornbeam, cedar, birch, hazel, beech, ash, privet, oak, plane tree, cypress, palm, from flowers, such as

ragweed, carnation, forsythia, sunflower, lupine, chamomile, lilac, passion flower, from grasses, such as quack grass, common bent, brome grass, Bermuda grass, sweet vernal grass, rye grass, or from other plants, such as opium poppy, pellitory, ribwort, tobacco, asparagus, mugwort, cress, etc.

Antigens derived from fungi, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may include antigens derived from, without being limited thereto, e.g. Alternia sp., Aspergillus sp., Beauveria sp., Candida sp., Cladosporium sp., Endothia sp., Curcularia sp., Embellisia sp., Epicoccum sp., Fusarium sp., Malassezia sp., Penicillum sp., Pleospora sp., Saccharomyces sp., etc.

Antigens derived from bacteria, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may include antigens derived from, without being limited thereto, e.g. Bacillus tetani, Staphylococcus aureus, Streptomyces griseus, etc.

c) Antibodies

According to a further embodiment, the nucleic acid (molecule), preferably an mRNA, of the at least one "complexed precomplexed nucleic acid" according to the present invention, may encode an antibody. According to the present invention, such an antibody may be selected from any antibody, e.g. any recombinantly produced or naturally occurring antibodies, known in the art, in particular antibodies suitable for therapeutic, diagnostic or scientific purposes, or antibodies which have been identified in relation to specific cancer diseases. Herein, the term "antibody" is used in its broadest sense and specifically covers monoclonal and polyclonal antibodies (including agonist, antagonist, and blocking or neutralizing antibodies) and antibody species with polyepitopic specificity. According to the invention, "antibody" typically comprises any antibody known in the art (e.g. IgM, IgD, IgG, IgA and IgE antibodies), such as naturally occurring antibodies, antibodies generated by immunization in a host organism, antibodies which were isolated and identified from naturally occurring antibodies or antibodies generated by immunization in a host organism and recombinantly produced by biomolecular methods known in the art, as well as chimeric antibodies, human

antibodies, humanized antibodies, bispecific antibodies, intrabodies, i.e. antibodies expressed in cells and optionally localized in specific cell compartments, and fragments and variants of the aforementioned antibodies. In general, an antibody consists of a light chain and a heavy chain both having variable and constant domains. The light chain consists of an N-terminal variable domain, V_L, and a C-terminal constant domain, C_L. In contrast, the heavy chain of the IgG antibody, for example, is comprised of an N-terminal variable domain, V_H, and three constant domains, C_H1, C_H2 und C_H3. Single chain antibodies may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention as well, preferably by a single-stranded RNA, more preferably by an mRNA.

According to a first alternative, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, preferably an mRNA, may encode a polyclonal antibody. In this context, the term, "polyclonal antibody" typically means mixtures of antibodies directed to specific antigens or immunogens or epitopes of a protein which were generated by immunization of a host organism, such as a mammal, e.g. including goat, cattle, swine, dog, cat, donkey, monkey, ape, a rodent such as a mouse, hamster and rabbit. Polyclonal antibodies are generally not identical, and thus usually recognize different epitopes or regions from the same antigen. Thus, in such a case, typically a mixture (a composition) of different nucleic acids, preferably mRNAs, will be used as the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, each nucleic acid encoding a specific (monoclonal) antibody being directed to specific antigens or immunogens or epitopes of a protein.

According to a further alternative, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, preferably an mRNA, may encode a monoclonal antibody. The term "monoclonal antibody" herein typically refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed to a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which

typically include different antibodies directed to different determinants (epitopes), each monoclonal antibody is directed to a single determinant on the antigen. For example, monoclonal antibodies as defined above may be made by the hybridoma method first described by Kohler and Milstein, Nature, 256:495 (1975), or may be made by recombinant DNA methods, e.g. as described in U.S. Pat. No. 4,816,567. "Monoclonal antibodies" may also be isolated from phage libraries generated using the techniques described in McCafferty *et al.*, Nature, 348:552-554 (1990), for example. According to Kohler and Milstein, an immunogen (antigen) of interest is injected into a host such as a mouse and B-cell lymphocytes produced in response to the immunogen are harvested after a period of time. The B-cells are combined with myeloma cells obtained from mouse and introduced into a medium which permits the B-cells to fuse with the myeloma cells, producing hybridomas. These fused cells (hybridomas) are then placed into separate wells of microtiter plates and grown to produce monoclonal antibodies. The monoclonal antibodies are tested to determine which of them are suitable for detecting the antigen of interest. After being selected, the monoclonal antibodies can be grown in cell cultures or by injecting the hybridomas into mice. However, for the purposes of the present invention, the peptide sequences of these monoclonal antibodies have to be sequenced and the nucleic acid sequences encoding these antibodies can be used as the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, which can be prepared according to procedures well known in the art.

For therapeutical purposes in humans, non-human monoclonal or polyclonal antibodies, such as murine antibodies may also be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention. However, such antibodies are typically only of limited use, since they generally induce an immune response by production of human antibodies directed to the said non-human antibodies, in the human body. Therefore, a particular non-human antibody can only be administered once to the human. To solve this problem, chimeric, humanized non-human and human antibodies are also envisaged as antibodies encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention. "Chimeric" antibodies, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the

present invention, are preferably antibodies in which the constant domains of an antibody described above are replaced by sequences of antibodies from other organisms, preferably human sequences. „Humanized" (non-human) antibodies, which may be also encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, are antibodies in which the constant and variable domains (except for the hypervariable domains) described above of an antibody are replaced by human sequences. According to another alternative, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may encode human antibodies, i.e. antibodies having only human sequences. Such human antibodies can be isolated from human tissues or from immunized non-human host organisms which are transgene for the human IgG gene locus, and RNA sequences may be prepared according to procedures well known in the art. Additionally, human antibodies can be provided by the use of a phage display.

In addition, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may encode bispecific antibodies. "Bispecific" antibodies in context of the invention are preferably antibodies which act as an adaptor between an effector and a respective target by two different $F_{a/b}$-domains, e.g. for the purposes of recruiting effector molecules such as toxins, drugs, cytokines etc., targeting effector cells such as CTL, NK cells, makrophages, granulocytes, etc. (see for review: Kontermann R.E., Acta Pharmacol. Sin, 2005, 26(1): 1-9). Bispecific antibodies as described herein are, in general, configured to recognize by two different $F_{a/b}$-domains, e.g. two different antigens, immunogens, epitopes, drugs, cells (or receptors on cells), or other molecules (or structures) as described above. Bispecificity means herewith that the antigen-binding regions of the antibodies are specific for two different epitopes. Thus, different antigens, immunogens or epitopes, etc. can be brought close together, what, optionally, allows a direct interaction of the two components. For example, different cells such as effector cells and target cells can be connected via a bispecific antibody. Encompassed, but not limited, by the present invention are antibodies or fragments thereof which bind, on the one hand, a soluble antigen as described herein, and, on the other hand, an antigen or receptor on the surface of a tumor cell.

According to the invention, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may also encode intrabodies, wherein these intrabodies may be antibodies as defined above. Since these antibodies are intracellular expressed antibodies, i.e. antibodies which are encoded by nucleic acid (molecules) localized in specific areas of the cell and also expressed there, such antibodies may be termed intrabodies.

Antibodies as encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may preferably comprise full-length antibodies, i.e. antibodies composed of the full heavy and full light chains, as described above. However, derivatives of antibodies such as antibody fragments, variants or adducts, may also be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention.

The nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may also encode antibody fragments selected from Fab, Fab', $F(ab')_2$, Fc, Facb, pFc', Fd and Fv fragments of the aforementioned (full-length) antibodies. In general, antibody fragments are known in the art. For example, an Fab ("fragment, antigen binding") fragment is composed of one constant and one variable domain of each of the heavy and the light chain. The two variable domains bind the epitope on specific antigens. The two chains are connected via a disulfide linkage. An scFv ("single chain variable fragment") fragment, for example, typically consists of the variable domains of the light and heavy chains. The domains are linked by an artificial linkage, in general a polypeptide linkage such as a peptide composed of 15-25 glycine, proline and/or serine residues.

According to a further embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may be an siRNA. An siRNA is of interest particularly in connection with the phenomenon of RNA interference. Attention was drawn to the phenomenon of RNA interference in the course of immunological research. In recent years, an RNA-based defence mechanism has been discovered, which occurs both in the kingdom of the fungi and in the plant and animal kingdom and acts as an "immune system of the genome". The system was originally

described in various species independently of one another, first in *C. elegans*, before it was possible to identify the underlying mechanisms of the processes as being identical: RNA-mediated virus resistance in plants, PTGS (posttranscriptional gene silencing) in plants, and RNA interference in eukaryotes are accordingly based on a common procedure. The *in vitro* technique of RNA interference (RNAi) is based on double-stranded RNA molecules (dsRNA), which trigger the sequence-specific suppression of gene expression (Zamore (2001) Nat. Struct. Biol. 9: 746-750; Sharp (2001) Genes Dev. 5:485-490: Hannon (2002) Nature 41: 244-251). In the transfection of mammalian cells with long dsRNA, the activation of protein kinase R and RnaseL brings about unspecific effects, such as, for example, an interferon response (Stark *et al.* (1998) Annu. Rev. Biochem. 67: 227-264; He and Katze (2002) Viral Immunol. 15: 95-119). Recently, dsRNA molecules have also been used *in vivo* (McCaffrey *et al.* (2002), Nature 418: 38-39; Xia *et al.* (2002), Nature Biotech. 20: 1006-1010; Brummelkamp *et al.* (2002), Cancer Cell 2: 243-247). Thus, an siRNA used for the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may comprise a (single- or) double stranded, preferably a double-stranded, RNA sequence with about 8 to 30 nucleotides, preferably 17 to 25 nucleotides, even more preferably from 20 to 25 and most preferably from 21 to 23 nucleotides. In principle, all the sections having a length of from 17 to 29, preferably from 19 to 25, most preferably from 21 to 23 base pairs that occur in the coding region of an RNA sequence as mentioned above can serve as target sequence for such an siRNA. Equally, siRNAs can also be directed against nucleotide sequences of a protein, particularly of regulatory proteins, which negatively regulate induction of an (innate or adaptive) immune response, that do not lie in the coding region, in particular in the 5' non-coding region of the RNA, for example, therefore, against non-coding regions of an RNA having a regulatory function. The target sequence of the siRNA, used as the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, can therefore lie in the translated and/or untranslated region of the nucleotide sequence of such a protein as defined above and/or in the region of its control elements. The target sequence of an siRNA as defined above can also lie in the overlapping region of untranslated and translated sequence; in particular, the target sequence can comprise at least one nucleotide upstream of the start triplet of the coding region of the RNA.

According to another embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may be an antisense RNA. In the context of the present invention, an antisense RNA is preferably a (single-stranded) RNA molecule transcribed off the coding, rather than the template, strand of a DNA, so that (preferably) the (entire) anti-sense mRNA sequence is complementary to the sense (messenger) RNA. An antisense RNA as defined herein typically forms a duplex between the sense and antisense RNA molecules and is thus capable to block transcription of the coding strand. An antisense RNA used as the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention can be directed against the nucleotide sequences, e.g. a (naturally occurring) mRNA or genomic sequence encoding a protein or peptide, which may be selected from any protein or peptide sequence suitable for that purpose. Preferably, the antisense RNA used herein as the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention comprises a length as defined above in general for RNA molecules, more preferably a length of 1000 to 5000, of 500 to 5000, of 5 to 5000, or of 5 to 1000, 5 to 500, 5 to 250, of 5 to 100, of 5 to 50 or of 5 to 30 nucleotides.

According to the present invention, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention as defined above, encoding a protein as defined above, e.g. a therapeutically active protein, antibody and/or antigen, may also encode fragments and/or variants of the aforementioned proteins, wherein the fragments and/or variants may have a sequence identity to one of the aforementioned proteins of at least 70%, 80% or 85%, preferably at least 90%, more preferably at least 95% and most preferably at least 99% over the whole length of the (coding) nucleic acid sequences encoding these proteins.

In the context of the present invention a fragment of such a protein, encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may preferably comprise a sequence of a protein as defined above, which is, with regard to its amino acid sequence (or its encoded nucleic acid sequence), N-terminally, C-terminally and/or intrasequentially truncated compared to the amino acid sequence of the original (native) protein (or its encoded nucleic acid sequence). Such truncation may thus occur either on the amino acid level or correspondingly on the nucleic

acid level. A sequence homology as defined above may therefore either refer to the entire (protein or nucleic acid) sequence or to the coding (nucleic acid) sequence of a protein as defined herein, encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention.

Fragments of a protein, encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may furthermore comprise a sequence of a protein as defined above, which has a length of about 6 to about 20 or even more amino acids, e.g. fragments as processed and presented by MHC class I molecules, preferably having a length of about 8 to about 10 amino acids, e.g. 8, 9, or 10, (or even 6, 7, 11, or 12 amino acids), or fragments as processed and presented by MHC class II molecules, preferably having a length of about 13 or more amino acids, e.g. 13, 14, 15, 16, 17, 18, 19, 20 or even more amino acids, wherein these fragments may be selected from any part of the amino acid sequence of such a protein. These fragments are typically recognized by T-cells in form of a complex consisting of the peptide fragment and an MHC molecule, i.e. the fragments are typically not recognized in their native form.

Fragments of a protein, encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may also comprise epitopes of those proteins. Epitopes (also called "antigen determinants") in the context of the present invention are typically fragments located on the outer surface of (native) proteins as defined herein, preferably having 5 to 15 amino acids, more preferably having 5 to 12 amino acids, even more preferably having 6 to 9 amino acids, which may be recognized by antibodies, i.e. in their native form. Such epitopes of proteins, encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may furthermore be selected from any of the herein mentioned variants of such proteins.

"Variants" of proteins, encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, preferably comprises a sequence, wherein nucleic acids of the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, are exchanged. Thereby, a protein may be generated, having an amino acid sequence which

differs from the original sequence in one or more mutation(s), such as one or more substituted, inserted and/or deleted amino acid(s). Preferably, these fragments and/or variants have the same or an improved biological function or specific activity compared to the full-length native protein as defined above.

The nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may also encode a protein as defined above, wherein the encoded amino acid sequence of the protein comprises a conservative amino acid substitution(s) compared to its physiological sequence. Those encoded amino acid sequences as well as their encoding nucleotide sequences in particular fall under the term variants as defined above. Substitutions in which amino acids which originate from the same class are exchanged for one another are called conservative substitutions. In particular, these are amino acids having aliphatic side chains, positively or negatively charged side chains, aromatic groups in the side chains or amino acids, the side chains of which can enter into hydrogen bridges, e.g. side chains which have a hydroxyl function. This means that e.g. an amino acid having a polar side chain is replaced by another amino acid having a likewise polar side chain, or, for example, an amino acid characterized by a hydrophobic side chain is substituted by another amino acid having a likewise hydrophobic side chain. Insertions and substitutions are possible, in particular, at those sequence positions which cause no modification to the three-dimensional structure or do not affect the binding region. Modifications to a three-dimensional structure by insertion(s) or deletion(s) can easily be determined e.g. using CD spectra (circular dichroism spectra) (Urry, 1985, Absorption, Circular Dichroism and ORD of Polypeptides, in: Modern Physical Methods in Biochemistry, Neuberger *et al.* (ed.), Elsevier, Amsterdam).

Preferred conservative amino acid substitutions of preferred groups of synonymous amino acid residues within the above meaning include, without being limited thereto:

Amino Acid	Synonymous Residue
Ser	Ser, Thr, Gly, Asn
Arg	Arg, Gln, Lys, Glu, His
Leu	Ile, Phe, Tyr, Met, Val, Leu
Pro	Gly, Ala, (Thr), Pro
Thr	Pro, Ser, Ala, Gly, His, Gln, Thr
Ala	Gly, Thr, Pro, Ala
Val	Met, Tyr, Phe, Ile, Leu, Val

Gly	Ala, (Thr), Pro, Ser, Gly
Ile	Met, Tyr, Phe, Val, Leu, Ile
Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
Cys	Ser, Thr, Cys
His	Glu, Lys, Gln, Thr, Arg, His
Gln	Glu, Lys, Asn, His, (Thr), Arg, Gln
Asn	Gln, Asp, Ser, Asn
Lys	Glu, Gln, His, Arg, Lys
Asp	Glu, Asn, Asp
Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

Furthermore, variants of proteins as defined above, which may be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, may also comprise those sequences, wherein nucleic acids of this nucleic acid (molecule) are exchanged according to the degeneration of the genetic code, without leading to an alteration of respective amino acid sequence of the antigen or antigenic protein, i.e. the amino acid sequence or at least part thereof may not differ from the original sequence in one or more mutation(s) within the above meaning.

In order to determine the percentage to which two sequences (nucleic acid sequences, e.g. (m)RNA (RNA or mRNA) sequences as defined herein, or amino acid sequences, preferably their encoded amino acid sequences, e.g. the amino acid sequences of the adjuvant proteins as defined above) are identical, the sequences can be aligned in order to be subsequently compared to one another. Therefore, e.g. gaps can be inserted into the sequence of the first sequence and the component at the corresponding position of the second sequence can be compared. If a position in the first sequence is occupied by the same component as is the case at a position in the second sequence, the two sequences are identical at this position. The percentage to which two sequences are identical is a function of the number of identical positions divided by the total number of positions. The percentage to which two sequences are identical can be determined using a mathematical algorithm. A preferred, but not limiting, example of a mathematical algorithm which can be used is the algorithm of Karlin *et al.* (1993), PNAS USA, 90:5873-5877 or Altschul *et al.* (1997), Nucleic Acids Res., 25:3389-3402. Such an algorithm is integrated in the BLAST program. Sequences which are identical to the sequences of the present invention to a certain extent can be identified by this program.

As mentioned above, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may occur as a mono-, di-, or even multicistronic nucleic acid (molecule), preferably an mRNA, i.e. a nucleic acid (molecule) which carries the coding sequences of at least one protein or peptide, e.g. of one, two or more proteins or peptides, as disclosed herein. Thus, the term "encoding at least one protein" may mean, without being limited thereto, that the at least one "complexed precomplexed nucleic acid", encodes at least one protein or peptide, preferably one, two, three or even more adjuvant proteins or peptides.

If the at least one protein is encoded by a monocistronic nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid", e.g. a monocistronic (m)RNA, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" may include various types of monocistronic nucleic acid(s) (molecules) coding for different proteins as defined herein, wherein each of the at least one nucleic acid molecules preferably encodes a (preferably different) protein as defined herein. In other words, a composition may contain several monocistronic nucleic acid(s) (molecules), wherein each of these monocistronic nucleic acid(s) (molecules) encodes for one (preferably different) protein, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more monocistronic nucleic acid(s) (molecules), wherein each of these monocistronic nucleic acid(s) (molecules) encodes for one (preferably different) protein. In any case, the at least one protein encoded thereby may be selected independently from any one of the proteins as defined above.

According to another particularly preferred embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may include at least one bi- or even multicistronic nucleic acid (molecule), which carries two or even more of the coding sequences of the at least one protein as defined herein. Such coding sequences of the at least one (preferably different) protein of the at least one bi- or even multicistronic nucleic acid (molecule) may be separated by at least one IRES (internal ribosomal entry site) sequence, as defined below. Thus, the term "encoding at least one (preferably different) protein" may mean, without being limited thereto, that the (bi- or even multicistronic) nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" may encode e.g. at least one, two, three, four, five, six, seven,

eight, nine, ten, etc. (preferably different) proteins of the above mentioned group of proteins. In any case, each of these proteins may be selected independently from one of the proteins as defined above. In this context, a so-called IRES (internal ribosomal entry site) sequence as defined above can function as a sole ribosome binding site, but it can also serve to provide a bi- or even multicistronic nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" which encodes several proteins which are to be translated by the ribosomes independently of one another. Examples of IRES sequences which can be used according to the invention are those from picornaviruses (e.g. FMDV), pestiviruses (CFFV), polioviruses (PV), encephalomyocarditis viruses (ECMV), foot and mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), mouse leukoma virus (MLV), simian immunodeficiency viruses (SIV) or cricket paralysis viruses (CrPV).

According to a further particularly preferred embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may furthermore include a mixture of at least one monocistronic nucleic acid (molecule) as defined above, and at least one bi- or even multicistronic nucleic acid (molecules) as defined above. The at least one monocistronic nucleic acid (molecule) and/or the at least one bi- or even multicistronic nucleic acid (molecule) preferably encode different proteins selected from the proteins as defined herein. However, the at least one monocistronic nucleic acid (molecule) and the at least one bi- or even multicistronic nucleic acid (molecule) may preferably also encode (in part) identical proteins. Use of a mixture of at least one monocistronic nucleic acid (molecule) as defined above, and at least one bi- or even multicistronic nucleic acid (molecule) or of a mixture of several at least one monocistronic nucleic acid(s) (molecules), wherein the at least one nucleic acid (molecule) encodes at least one (preferably different) protein as defined herein may be advantageous e.g. for a staggered, e.g. time dependent, administration of the "complexed precomplexed nucleic acid" according to the present invention or a composition thereof to a patient in need thereof. The different nucleic acid(s) (molecules) "complexed precomplexed nucleic acid" according to the present invention, may be e.g. contained in (different parts of) a kit of parts composition and may be administered together or separately.

According to one embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may be in the form of a modified nucleic acid (molecule), preferably a modified mRNA, wherein any modification, as defined herein, may be introduced into the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention. Modifications as defined herein preferably lead to a stabilized nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention. Some modifications of the nucleic acid (molecule) may be, dependent on the type of nucleic acid (molecule), more suitable for a nucleic acid moleucla, e.g. a DNA or RNA in general, or, e.g. in the case of GC-modified (m)RNA or mRNA sequences, be more suitable for a coding RNA, preferably an (m)RNA and/or mRNA as defined above.

According to one specific embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may thus be provided as a "stabilized nucleic acid (molecule)", that is to say as a nucleic acid molecule, preferably an mRNA, that is essentially resistant to *in vivo* degradation (e.g. by an exo- or endo-nuclease). Such stabilization can be effected, for example, by a modified phosphate backbone of the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention. A backbone modification in connection with the present invention is a modification in which phosphates of the backbone of the nucleotides contained in the nucleic acid (molecule) are chemically modified. Nucleotides that may be preferably used in this connection contain e.g. a phosphorothioate-modified phosphate backbone, preferably at least one of the phosphate oxygens contained in the phosphate backbone being replaced by a sulfur atom. Stabilized nucleic acid(s) (molecules) may further include, for example: non-ionic phosphate analogues, such as, for example, alkyl and aryl phosphonates, in which the charged phosphonate oxygen is replaced by an alkyl or aryl group, or phosphodiesters and alkylphosphotriesters, in which the charged oxygen residue is present in alkylated form. Such backbone modifications typically include, without implying any limitation, modifications from the group consisting of methylphosphonates, phosphoramidates and phosphorothioates (e.g. cytidine-5'-O-(1-thiophosphate)).

The nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may additionally or alternatively also contain sugar modifications. A sugar modification in connection with the present invention is a chemical modification of the sugar of the nucleotides of the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention and typically includes, without implying any limitation, sugar modifications selected from the group consisting of 2'-deoxy-2'-fluoro-oligoribonucleotide (2'-fluoro-2'-deoxycytidine-5'-triphosphate, 2'-fluoro-2'-deoxyuridine-5'-triphosphate), 2'-deoxy-2'-deamine oligoribonucleotide (2'-amino-2'-deoxycytidine-5'-triphosphate, 2'-amino-2'-deoxyuridine-5'-triphosphate), 2'-O-alkyl oligoribonucleotide, 2'-deoxy-2'-C-alkyl oligoribonucleotide (2'-O-methylcytidine-5'-triphosphate, 2'-methyluridine-5'-triphosphate), 2'-C-alkyl oligoribonucleotide, and isomers thereof (2'-aracytidine-5'-triphosphate, 2'-arauridine-5'-triphosphate), or azidotriphosphate (2'-azido-2'-deoxycytidine-5'-triphosphate, 2'-azido-2'-deoxyuridine-5'-triphosphate).

The nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may additionally or alternatively also contain at least one base modification, which is preferably suitable for increasing the expression of the protein encoded by the nucleic acid (molecule) significantly as compared with the unaltered, i.e. natural (= native) nucleic acid sequence (molecule). Significant in this case means an increase in the expression of the protein by at least 20%, preferably at least 30%, 40%, 50% or 60%, more preferably by at least 70%, 80%, 90% or even 100% and most preferably by at least 150%, 200% or even 300% or more. In connection with the present invention, a nucleotide having such a base modification is preferably selected from the group of the base-modified nucleotides consisting of 2-amino-6-chloropurineriboside-5'-triphosphate, 2-aminoadenosine-5'-triphosphate, 2-thiocytidine-5'-triphosphate, 2-thiouridine-5'-triphosphate, 4-thiouridine-5'-triphosphate, 5-aminoallylcytidine-5'-triphosphate, 5-aminoallyluridine-5'-triphosphate, 5-bromocytidine-5'-triphosphate, 5-bromouridine-5'-triphosphate, 5-iodocytidine-5'-triphosphate, 5-iodouridine-5'-triphosphate, 5-methylcytidine-5'-triphosphate, 5-methyluridine-5'-triphosphate, 6-azacytidine-5'-triphosphate, 6-azauridine-5'-triphosphate, 6-chloropurineriboside-5'-triphosphate, 7-deazaadenosine-5'-triphosphate, 7-deazaguanosine-5'-triphosphate, 8-azaadenosine-5'-triphosphate, 8-azidoadenosine-5'-triphosphate, benzimidazole-riboside-5'-triphosphate,

N1-methyladenosine-5'-triphosphate, N1-methylguanosine-5'-triphosphate, N6-methyladenosine-5'-triphosphate, O6-methylguanosine-5'-triphosphate, pseudouridine-5'-triphosphate, or puromycin-5'-triphosphate, xanthosine-5'-triphosphate. Particular preference is given to nucleotides for base modifications selected from the group of base-modified nucleotides consisting of 5-methylcytidine-5'-triphosphate, 7-deazaguanosine-5'-triphosphate, 5-bromocytidine-5'-triphosphate, and pseudouridine-5'-triphosphate.

According to another embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention can likewise be modified (and preferably stabilized) by introducing modified nucleotides containing modifications of their ribose or base moieties. Generally, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may contain any native (= naturally occurring) nucleotide, e.g. guanosine, thymidine, uracil, adenosine, and cytosine or an analogue thereof. In this connection, nucleotide analogues are defined as non-natively occurring variants of naturally occurring nucleotides. Accordingly, analogues are chemically derivatized nucleotides with non-natively occurring functional groups, which are preferably added to or deleted from the naturally occurring nucleotide or which substitute the naturally occurring functional groups of a nucleotide. Accordingly, each component of the naturally occurring nucleotide may be modified, namely the base component, the sugar (ribose) component and/or the phosphate component forming the oligonucleotide's backbone (see above). Modifications of the, preferably of the base component, may comprise e.g. analogues of guanosine, uracil, adenosine, and cytosine, which include, without implying any limitation, any naturally occurring or non-naturally occurring guanosine, uracil, adenosine, thymidine or cytosine that has been altered chemically, for example by acetylation, methylation, hydroxylation, etc., including 1-methyl-adenosine, 1-methyl-guanosine, 1-methyl-inosine, 2,2-dimethyl-guanosine, 2,6-diaminopurine, 2'-amino-2'-deoxyadenosine, 2'-amino-2'-deoxycytidine, 2'-amino-2'-deoxyguanosine, 2'-amino-2'-deoxyuridine, 2-amino-6-chloropurineriboside, 2-aminopurine-riboside, 2'-araadenosine, 2'-aracytidine, 2'-arauridine, 2'-azido-2'-deoxyadenosine, 2'-azido-2'-deoxycytidine, 2'-azido-2'-deoxyguanosine, 2'-azido-2'-deoxyuridine, 2-chloroadenosine, 2'-fluoro-2'-deoxyadenosine, 2'-fluoro-2'-deoxycytidine, 2'-fluoro-2'-deoxyguanosine, 2'-fluoro-2'-deoxyuridine, 2'-fluorothymidine, 2-methyl-adenosine, 2-methyl-guanosine, 2-methyl-thio-N6-isopenenyl-adenosine, 2'-O-methyl-2-

aminoadenosine, 2'-O-Methyl-2'-deoxyadenosine, 2'-O-methyl-2'-deoxycytidine, 2'-O-methyl-2'-deoxyguanosine, 2'-O-methyl-2'-deoxyuridine, 2'-O-methyl-5-methyluridine, 2'-O-methylinosine, 2'-O-methylpseudouridine, 2-thiocytidine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 4-thiouridine, 5-(carboxyhydroxymethyl)-uracil, 5,6-dihydrouridine, 5-aminoallylcytidine, 5-Aminoallyl-deoxy-uridine, 5-bromouridine, 5-carboxymehtylaminomethyl-2-thio-uracil, 5-carboxymethylamonomethyl-uracil, 5-chloro-ara-cytosine, 5-fluoro-uridine, 5-iodouridine, 5-methoxycarbonylmethyl-uridine, 5-methoxy-uridine, 5-methyl-2-thio-uridine, 6-azacytidine, 6-azauridine, 6-chloro-7-deaza-guanosine, 6-chloropurineriboside, 6-mercapto-guanosine, 6-methyl-mercaptopurine-riboside, 7-deaza-2'-deoxy-guanosine, 7-deazaadenosine, 7-methyl-guanosine, 8-azaadenosine, 8-bromo-adenosine, 8-bromo-guanosine, 8-mercapto-guanosine, 8-oxoguanosine, benzimidazole-riboside, beta-D-mannosyl-queosine, dihydro-uracil, inosine, N1-methyladenosine, N6-([6-aminohexyl]carbamoylmethyl)-adenosine, N6-isopentenyl-adenosine, N6-methyl-adenosine, N7-methyl-xanthosine, N-uracil-5-oxyacetic acid methyl ester, puromycin, queosine, uracil-5-oxyacetic acid, uracil-5-oxyacetic acid methyl ester, wybutoxosine, xanthosine, and xylo-adenosine. Further modifications, preferably of the sugar component, may comprise analogs such as e.g. 2'-Deoxy-2'-fluoro-oligoribonucleotide (e.g. 2'-fluoro-2'-deoxycytidine-triphosphate, 2'-fluoro-2'-deoxyuridine-triphosphate), 2'-deoxy-2'-deamine-oligoribonucleotide (e.g. 2'-amino-2'-deoxycytidine-5'-triphosphate, 2'-amino-2'-deoxyuridine-5'-triphosphate), 2'-O-alkyl-oligoribonucleotide, 2'-deoxy-2'-C-alkyl-oligoribonucleotide (e.g. 2'-O-methylcytidine-5'-triphosphate, 2'-methyluridine-5'-triphosphate), 2'-C-alkyl oligoribonucleotide, isomers thereof (e.g. 2'-aracytidine-5'-triphosphate, 2'-arauridine-5'-triphosphate), or azido-triphosphate (e.g. 2'-azido-2'-deoxycytidine-triphosphate, 2'-azido-2'-deoxyuridine-triphosphate). The preparation of such analogues is known to a 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. In the case of an analogue as described above, particular preference is given according to the invention to those analogues that do not interfere with a further modification that has been introduced into the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention.

According to a particularly specific embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention may comprise between 0.1% and 100% (modified) nucleotides selected from (modified) nucleotides as defined above with respect to the non-modified nucleic acid (molecule), wherein preferably between 0.1% and 100% of each natively occurring non-modified ATP, GTP, CTP, UTP (and/or TTP) nucleotide of a nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, or of its corresponding DNA-template, may be modified using a corresponding modified nucleotide as defined above, more preferably between 0,1% and 20%, between 10% and 30%, between 20% and 40%, between 30% and 50%, between 40% and 60%, between 50% and 70%, between 60% and 80%, between 70% and 90%, or between 80% and 100% or at least 10%, more preferably at least 30%, more preferably at least 40%, more preferably at least 60%, more preferably at least 70%, more preferably at least 80% and more preferably at least 90% and most preferably 100% of each natively occurring non-modified ATP, GTP CTP, UTP (and/or TTP) nucleotide of the non-modified nucleic acid (molecule).

According to a particular embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention can contain a lipid modification, i.e. a lipid-modified nucleic acid (molecule). Such a lipid-modified nucleic acid (molecule) typically comprises a nucleic acid (molecule) as defined herein for the at least one "complexed precomplexed nucleic acid", at least one linker covalently linked with that nucleic acid (molecule), and at least one lipid covalently linked with the respective linker. Alternatively, such a lipid-modified nucleic acid (molecule) comprises a (at least one) nucleic acid (molecule) as defined herein and at least one (bifunctional) lipid covalently linked (without a linker) with that nucleic acid (molecule). According to a third alternative, the lipid-modified nucleic acid (molecule) comprises a nucleic acid (molecule) as defined herein, at least one linker covalently linked with that nucleic acid (molecule), and at least one lipid covalently linked with the respective linker, and also at least one (bifunctional) lipid covalently linked (without a linker) with that nucleic acid (molecule).

The lipid of a lipid-modified nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention (complexed or covalently bound thereto) is typically a lipid or a lipophilic residue that preferably is itself biologically

active. Such lipids preferably include natural substances or compounds such as, for example, vitamins, e.g. alpha-tocopherol (vitamin E), including RRR-alpha-tocopherol (formerly D-alpha-tocopherol), L-alpha-tocopherol, the racemate D,L-alpha-tocopherol, vitamin E succinate (VES), or vitamin A and its derivatives, e.g. retinoic acid, retinol, vitamin D and its derivatives, e.g. vitamin D and also the ergosterol precursors thereof, vitamin E and its derivatives, vitamin K and its derivatives, e.g. vitamin K and related quinone or phytol compounds, or steroids, such as bile acids, for example cholic acid, deoxycholic acid, dehydrocholic acid, cortisone, digoxygenin, testosterone, cholesterol or thiocholesterol. Further lipids or lipophilic residues used within the scope of the present invention include, without implying any limitation, polyalkylene glycols (Oberhauser *et al.*, Nucl. Acids Res., 1992, 20, 533), aliphatic groups such as, for example, C1-C20-alkanes, C1-C20-alkenes or C1-C20-alkanol compounds, etc., such as, for example, dodecanediol, hexadecanol or undecyl residues (Saison-Behmoaras *et al.*, EMBO J, 1991, 10, 111; Kabanov *et al.*, FEBS Lett., 1990, 259, 327; Svinarchuk *et al.*, Biochimie, 1993, 75, 49), phospholipids such as, for example, phosphatidylglycerol, diacylphosphatidylglycerol, phosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, di-hexadecyl-rac-glycerol, sphingolipids, cerebrosides, gangliosides, or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, Tetrahedron Lett., 1995, 36, 3651; Shea *et al.*, Nucl. Acids Res., 1990, 18, 3777), polyamines or polyalkylene glycols, such as, for example, polyethylene glycol (PEG) (Manoharan *et al.*, Nucleosides & Nucleotides, 1995, 14, 969), hexaethylene glycol (HEG), palmitin or palmityl residues (Mishra *et al.*, Biochim. Biophys. Acta, 1995, 1264, 229), octadecylamines or hexylamino-carbonyl-oxycholesterol residues (Crooke *et al.*, J. Pharmacol. Exp. Ther., 1996, 277, 923), and also waxes, terpenes, alicyclic hydrocarbons, saturated and mono- or poly-unsaturated fatty acid residues, etc.

The nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, may likewise be stabilized in order to prevent degradation of the nucleic acid molecule *in vivo* by various approaches. In comparison with DNA, RNA is considerably less stable in solution, which is substantially due to RNA-degrading enzymes, so-called RNases (ribonucleases). Even the smallest ribonuclease contaminations are sufficient to degrade RNA completely in solution. Such RNase contaminations can generally be removed only by

special treatment, in particular with diethyl pyrocarbonate (DEPC). Accordingly, the natural degradation of mRNA in the cytoplasm of cells is very finely regulated. A number of mechanisms are known in this connection in the prior art. Thus, the terminal structure is typically of critical importance for an mRNA *in vivo*. At the 5' end of naturally occurring mRNAs there is usually a so-called "cap structure" (a modified guanosine nucleotide) and at the 3' end a sequence of up to 200 adenosine nucleotides (the so-called poly-A tail).

The nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as a RNA or mRNA,, can therefore be stabilized against degradation by RNases by the addition of a so-called "5' cap" structure. Particular preference is given in this connection to a m7G(5')ppp (5'(A,G(5')ppp(5')A or G(5')ppp(5')G as the 5' cap" structure. However, such a modification is introduced only if a modification, for example a lipid modification, has not already been introduced at the 5' end of the nucleic acid (molecule) or if the modification does not interfere with the desired immunogenic properties of the (unmodified or chemically modified) nucleic acid (molecule).

The nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, may contain, according to a further preferred embodiment of the present invention, a poly-A tail on the 3' terminus of typically about 10 to 200 adenosine nucleotides, preferably about 10 to 100 adenosine nucleotides, more preferably about 20 to 100 adenosine nucleotides or even more preferably about 40 to 80 adenosine nucleotides, e.g. 70 nucleotides.

The nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, may additionally or alternatively contain a poly-C tail on the 3' terminus of typically about 10 to 200 cytosine nucleotides, preferably about 10 to 100 cytosine nucleotides, more preferably about 20 to 70 cytosine nucleotides or even more preferably about 20 to 60 or even 10 or 20 to 40 cytosine nucleotides, e.g. 30 nucleotides.

According to another embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if

provided as an RNA or mRNA, may be modified, and thus stabilized, by modifying the G/C content of the nucleic acid (molecule), preferably of the coding region of the nucleic acid (molecule).

According to one specific embodiment, the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, may be stabilized by modifying the G/C content of the coding region of the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention.

In a particularly preferred embodiment of the present invention, the G/C content of the coding region of the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, is altered, preferably increased, compared to the G/C content of the coding region of the corresponding non-modified nucleic acid (molecule) (in the following "native" nucleic acid). In this context, the encoded amino acid sequence of this G/C-increased nucleic acid (molecule) is preferably not altered compared to the corresponding native nucleic acid (molecule). Such alteration of the GC-sequence may be termed in the following "GC-stabilization".

This G/C-stabilization of the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, is based on the fact that the sequence of any nucleic acid region to be translated is important for efficient translation of that nucleic acid (molecule). Thus, the sequence of various nucleotides is important. In particular, sequences having an increased G (guanosine)/C (cytosine) content are more stable than sequences having an increased A (adenosine)/U (uracil) content. According to the invention, the codons the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, are therefore varied compared to its native nucleic acid (molecule) sequence, while retaining the translated amino acid sequence, such that they include an increased amount of G/C nucleotides. In respect to the fact that several codons code for one and the same amino acid (so-called degeneration of the genetic code), the most favorable codons for the stability can be

determined (so-called alternative codon usage).

Depending on the amino acid to be encoded by the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, there are various possibilities for G/C-modification of the nucleic acid (molecule) sequence, compared to its native sequence. In the case of amino acids which are encoded by codons which contain exclusively G or C nucleotides, no G/C-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) require no G/C-modification, since no A or U is present.

In contrast, codons which contain A and/or U nucleotides can be G/C-modified by substitution of other codons which code for the same amino acids but contain no A and/or U. Examples of these are:

the codons for Pro can be G/C-modified from CCU or CCA to CCC or CCG;
the codons for Arg can be G/C-modified from CGU or CGA or AGA or AGG to CGC or CGG;
the codons for Ala can be G/C-modified from GCU or GCA to GCC or GCG;
the codons for Gly can be G/C-modified from GGU or GGA to GGC or GGG.

In other cases, although A or U nucleotides cannot be eliminated from the codons, it is however possible to decrease the A and U content by using codons which contain a lower content of A and/or U nucleotides. Examples of these are:

the codons for Phe can be G/C-modified from UUU to UUC;
the codons for Leu can be G/C-modified from UUA, UUG, CUU or CUA to CUC or CUG;
the codons for Ser can be G/C-modified from UCU or UCA or AGU to UCC, UCG or AGC;
the codon for Tyr can be G/C-modified from UAU to UAC;
the codon for Cys can be G/C-modified from UGU to UGC;
the codon for His can be G/C-modified from CAU to CAC;
the codon for Gln can be G/C-modified from CAA to CAG;
the codons for Ile can be G/C-modified from AUU or AUA to AUC;

the codons for Thr can be G/C-modified from ACU or ACA to ACC or ACG;
the codon for Asn can be G/C-modified from AAU to AAC;
the codon for Lys can be G/C-modified from AAA to AAG;
the codons for Val can be G/C-modified from GUU or GUA to GUC or GUG;
the codon for Asp can be G/C-modified from GAU to GAC;
the codon for Glu can be G/C-modified from GAA to GAG;
the stop codon UAA can be G/C-modified to UAG or UGA.

In the case of the codons for Met (AUG) and Trp (UGG), on the other hand, there is no possibility of sequence modification.

The substitutions listed above can be used either individually or in all possible combinations to increase the G/C content of the nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, compared to its particular native nucleic acid (molecule) sequence. Thus, for example, all codons for Thr occurring in the native sequence can be G/C-modified to ACC (or ACG). Preferably, however, for example, combinations of the above substitution possibilities are used:

substitution of all codons coding for Thr in the non-modified sequence (native RNA) to ACC (or ACG) and
substitution of all codons originally coding for Ser to UCC (or UCG or AGC);
substitution of all codons coding for Ile in the original sequence to AUC and
substitution of all codons originally coding for Lys to AAG and
substitution of all codons originally coding for Tyr to UAC;
substitution of all codons coding for Val in the original sequence to GUC (or GUG) and
substitution of all codons originally coding for Glu to GAG and
substitution of all codons originally coding for Ala to GCC (or GCG) and
substitution of all codons originally coding for Arg to CGC (or CGG);
substitution of all codons coding for Val in the original sequence to GUC (or GUG) and
substitution of all codons originally coding for Glu to GAG and
substitution of all codons originally coding for Ala to GCC (or GCG) and
substitution of all codons originally coding for Gly to GGC (or GGG) and

substitution of all codons originally coding for Asn to AAC;
substitution of all codons coding for Val in the original sequence to GUC (or GUG) and
substitution of all codons originally coding for Phe to UUC and
substitution of all codons originally coding for Cys to UGC and
substitution of all codons originally coding for Leu to CUG (or CUC) and
substitution of all codons originally coding for Gln to CAG and
substitution of all codons originally coding for Pro to CCC (or CCG); etc.

Preferably, the G/C content of the coding region of the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, is increased by at least 7%, more preferably by at least 15%, particularly preferably by at least 20%, compared to the G/C content of the coding region of the native nucleic acid (molecule) which codes for a protein as defined herein. According to a specific embodiment at least 60%, more preferably at least 70 %, even more preferably at least 80% and most preferably at least 90%, 95% or even 100% of the substitutable codons in the region coding for a protein as defined herein or the whole sequence of the native nucleic acid (molecule) sequence are substituted, thereby increasing the GC/content of said sequence.

In this context, it is particularly preferable to increase the G/C content of the native nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, to the maximum (i.e. 100% of the substitutable codons of the native nucleic acid (molecule) sequence), in particular in the region coding for a protein as defined herein.

According to the invention, a further preferred modification of the nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, is based on the finding that the translation efficiency is also determined by a different frequency in the occurrence of tRNAs in cells. Thus, if so-called "rare codons" are present in a native RNA sequence to an increased extent, the corresponding G/C-stabilized or native RNA sequence may be translated to a significantly poorer degree than in the case, where codons coding for relatively "frequent" tRNAs are present.

According to the invention, the region in the nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, which code for a protein as defined herein, is preferably GC-stabilized compared to the corresponding region of the respective native nucleic acid (molecule) such that at least one codon of the native sequence which codes for a tRNA which is relatively rare in the cell is exchanged for a codon which codes for a tRNA which is relatively frequent in the cell and carries the same amino acid as the relatively rare tRNA. By this modification, the native nucleic acid (molecule) sequences are GC-stabilized such that codons for which frequently occurring tRNAs are available are inserted. In other words, according to the invention, by this modification all codons of the native sequence which code for a tRNA which is relatively rare in the cell can in each case be exchanged for a codon which codes for a tRNA which is relatively frequent in the cell and which, in each case, carries the same amino acid as the relatively rare tRNA.

Which tRNAs occur relatively frequently in the cell and which, in contrast, occur relatively rarely is known to a person skilled in the art; cf. e.g. Akashi, Curr. Opin. Genet. Dev. 2001, 11(6): 660-666. The codons which use for the particular amino acid the tRNA which occurs the most frequently, e.g. the Gly codon, which uses the tRNA which occurs the most frequently in the (human) cell, are particularly preferred.

According to the invention, it is particularly preferable to link the sequential G/C content which is increased, in particular maximized, in the GC-stabilized nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, with the "frequent" codons without modifying the amino acid sequence of the protein encoded by the coding region of the native nucleic acid (molecule). This preferred embodiment allows provision of a particularly efficiently translated and GC-stabilized nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention.

The determination of the necessary (and/or possible) GC modification of the nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, particularly if provided as an RNA or mRNA, as described above

(increased G/C content; exchange of tRNAs), can be carried out using the computer program as explained in WO 02/098443 - the disclosure content of which is included in its full scope in the present invention. Using this computer program, the nucleotide sequence of any desired (coding) nucleic acid (molecule) used for the at least one "complexed precomplexed nucleic acid" according to the present invention can be GC-stabilized with the aid of the genetic code or the degenerative nature thereof such that a maximum G/C content results. In combination with the use of codons which code for tRNAs occurring as frequently as possible in the cell, the amino acid sequence coded by the GC-stabilized nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention is preferably not further modified compared to their native RNA sequence. Alternatively, it is also possible to modify only the G/C content or only the codon usage compared to the native sequence. The source code in Visual Basic 6.0 (development environment used: Microsoft Visual Studio Enterprise 6.0 with Servicepack 3) is also described in WO 02/098443.

In a further preferred embodiment of the present invention, the A/U content in the environment of the ribosome binding site of an (optionally already GC-stabilized) nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, is increased compared to the A/U content in the environment of the ribosome binding site of its particular native nucleic acid (molecule) sequence. This modification (an increased A/U content around the ribosome binding site) increases the efficiency of ribosome binding to the nucleic acid (molecule) sequence. An effective binding of the ribosomes to the ribosome binding site (Kozak sequence: GCCGCCACCAUGG (SEQ ID NO: 8), the AUG forms the start codon) in turn has the effect of an efficient translation of the RNA sequence as defined herein.

According to a further embodiment of the present invention nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention may be further modified with respect to potentially destabilizing sequence elements. Particularly, the coding region and/or the 5' and/or 3' untranslated region of the nucleic acid (molecule) sequence as defined herein may be further modified compared to the particular native nucleic acid (molecule) sequence such that is contains no destabilizing sequence elements, the coded amino acid sequence of the nucleic acid (molecule)

sequence as defined herein preferably not being modified compared to its particular native nucleic acid (molecule) sequence. It is known that, for example, in sequences of eukaryotic RNAs destabilizing sequence elements (DSE) occur, to which signal proteins bind and regulate enzymatic degradation of RNA *in vivo*. For further stabilization of the nucleic acid (molecule) sequence as defined herein, optionally in the region which encodes for a protein, one or more such further modifications compared to the corresponding region of the native nucleic acid (molecule) sequence can therefore be carried out, so that no or substantially no destabilizing sequence elements are contained therein. According to the invention, DSEs present in the untranslated regions (3'- and/or 5'-UTR) can also be eliminated from a nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention by such further modifications.

Such destabilizing sequences are e.g. AU-rich sequences (AURES), which occur in 3'-UTR sections of numerous unstable RNAs (Caput *et al.*, Proc. Natl. Acad. Sci. USA 1986, 83: 1670 to 1674). A nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" as defined herein, is therefore preferably (further) modified compared to the native nucleic acid (molecule) sequence such that the modified nucleic acid (molecule) sequence contains no such destabilizing sequences. This also applies to those sequence motifs which are recognized by possible endonucleases, e.g. the sequence GAACAAG, which is contained in the 3'-UTR segment of the gene which codes for the transferrin receptor (Binder *et al.*, EMBO J. 1994, 13: 1969 to 1980). These sequence motifs are also preferably removed according to the invention in the nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention.

Also preferably according to the invention, the nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, preferably if provided as an mRNA, has, in a further modified form at least one IRES as defined above and/or at least one 5' and/or 3' stabilizing sequence, e.g. to enhance ribosome binding or to allow expression of different encoded proteins, e.g., antibodies, therapeutically active proteins or antigens, as defined above, located on an at least one (bi- or even multicistronic) nucleic acid (molecule) as defined above.

According to the invention, the nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, may furthermore have at least one (additional) 5' and/or 3' stabilizing sequence. These stabilizing sequences in the 5' and/or 3' untranslated regions have the effect of increasing the half-life of the nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" as defined herein in the cytosol. These stabilizing sequences can have 100% sequence homology to naturally occurring sequences which occur in viruses, bacteria and eukaryotes, but can also be partly or completely synthetic. The untranslated sequences (UTR) of the globin gene, e.g. from *Homo sapiens* or *Xenopus laevis* may be mentioned as an example of stabilizing sequences which can be used in the present invention for a further stabilized nucleic acid (molecule) sequence as defined herein. Another example of a stabilizing sequence has the general formula (C/U)CCAN$_x$CCC(U/A)Py$_x$UC(C/U)CC (SEQ ID NO: 9), which is contained in the 3'UTR of the very stable RNA which codes for globin, (I)-collagen, 15-lipoxygenase or for tyrosine hydroxylase (cf. Holcik *et al.*, Proc. Natl. Acad. Sci. USA 1997, 94: 2410 to 2414). Such stabilizing sequences can of course be used individually or in combination with one another and also in combination with other stabilizing sequences known to a person skilled in the art. A nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention is therefore preferably present as globin UTR (untranslated regions)-stabilized nucleic acid (molecule).

Any of the above modifications may be applied to a nucleic acid (molecule) sequence of the at least one "complexed precomplexed nucleic acid" according to the present invention, and may be, if suitable or necessary, be combined with each other in any combination, provided, these combinations of modifications do not interfere with each other in the respective modified nucleic acid (molecule). A person skilled in the art will be able to take his choice accordingly.

According to the invention, a nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" as defined herein may be prepared using any naturally or synthetic DNA or RNA sequence available in the art as a template, i.e. any suitable (desoxy)ribonucleic acid. Such naturally or synthetic DNA or RNA sequences may be

obtained from any synthetic or naturally occurring source, which is available to a skilled person, e.g. may be derived from a protein or peptide library or may be transcribed from a nucleic acid library, such as a cDNA library, or may be obtained from any living or dead tissue, from a sample obtained from e.g. a human, animal or bacterial source. Alternatively, a nucleic acid (molecule) as defined herein may be prepared synthetically by methods known to a person skilled in the art, e.g., by solid phase synthesis or any other suitable method for preparing nucleic acid sequences, particularly methods suitable for preparing mRNA sequences. Furthermore, substitutions, additions or eliminations of nucleotides or bases in these sequences are preferably carried out using a DNA matrix for preparation of a RNA or mRNA as defined herein or by techniques of the well known site directed mutagenesis or with an oligonucleotide ligation strategy (see e.g. Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 3rd ed., Cold Spring Harbor, NY, 2001). The modification(s) of a nucleic acid (molecule) of the at least one "complexed precomplexed nucleic acid" as defined herein can also be introduced into the nucleic acid (molecule) sequence by means of further methods known to a person skilled in the art. Suitable methods are, for example, synthesis methods using (automatic or semi-automatic) oligonucleotide synthesis devices, biochemical methods, such as, for example, *in vitro* transcription methods, etc. In this connection there can preferably be used in the case of (relatively short) sequences, whose length generally does not exceed from 50 to 100 nucleotides, synthesis methods using (automatic or semi-automatic) oligonucleotide synthesis devices as well as *in vitro* transcription methods. In the case of (relatively long) sequences, for example sequences having a length of more than 50 to 100 nucleotides, biochemical methods are preferably suitable, such as, for example, *in vitro* transcription methods. However, even longer base-modified nucleic acid molecules may be synthesized synthetically by coupling various synthesized fragments covalently.

According to a further embodiment, the present invention also provides a pharmaceutical composition, comprising the at least one "complexed precomplexed nucleic acid" as defined according to the present invention and optionally a pharmaceutically acceptable carrier and/or vehicle.

As a first ingredient, the inventive pharmaceutical composition comprises the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, i.e.

at least one complexed precomplexed nucleic acid (molecule), e.g. an mRNA, which has been precomplexed in a first step with (a high-molecular) PEI in an N/P ratio between 0.05 and 2, preferably in an N/P ratio between 0.1 and 1 and have been further complexed in a second step with a cationic or polycationic compound.

Furthermore, the inventive pharmaceutical composition may comprise a pharmaceutically acceptable carrier and/or vehicle. In the context of the present invention, a pharmaceutically acceptable carrier typically includes the liquid or non-liquid basis of the inventive inventive pharmaceutical composition. If the inventive pharmaceutical composition is provided in liquid form, the carrier will typically be pyrogen-free water; isotonic saline or buffered (aqueous) solutions, e.g phosphate, citrate etc. buffered solutions. Particularly for injection of the inventive inventive pharmaceutical composition, water or preferably a buffer, more preferably an aqueous buffer, may be used, containing a sodium salt, preferably at least 50 mM of a sodium salt, a calcium salt, preferably at least 0.01 mM of a calcium salt, and optionally a potassium salt, preferably at least 3 mM of a potassium salt. According to a preferred embodiment, the sodium, calcium and, optionally, potassium salts may occur in the form of their halogenides, e.g. chlorides, iodides, or bromides, in the form of their hydroxides, carbonates, hydrogen carbonates, or sulfates, etc. Without being limited thereto, examples of sodium salts include e.g. NaCl, NaI, NaBr, Na_2CO_3, $NaHCO_3$, Na_2SO_4, examples of the optional potassium salts include e.g. KCl, KI, KBr, K_2CO_3, $KHCO_3$, K_2SO_4, and examples of calcium salts include e.g. $CaCl_2$, CaI_2, $CaBr_2$, $CaCO_3$, $CaSO_4$, $Ca(OH)_2$. Furthermore, organic anions of the aforementioned cations may be contained in the buffer. According to a more preferred embodiment, the buffer suitable for injection purposes as defined above, may contain salts selected from sodium chloride (NaCl), calcium chloride ($CaCl_2$) and optionally potassium chloride (KCl), wherein further anions may be present additional to the chlorides. $CaCl_2$ can also be replaced by another salt like KCl. Typically, the salts in the injection buffer are present in a concentration of at least 50 mM sodium chloride (NaCl), at least 3 mM potassium chloride (KCl) and at least 0,01 mM calcium chloride ($CaCl_2$). The injection buffer may be hypertonic, isotonic or hypotonic with reference to the specific reference medium, i.e. the buffer may have a higher, identical or lower salt content with reference to the specific reference medium, wherein preferably such concentrations of the afore mentioned salts may be used, which do not lead to damage of cells due to osmosis or other concentration effects. Reference media are e.g. liquids

occurring in "*in vivo*" methods, such as blood, lymph, cytosolic liquids, or other body liquids, or e.g. liquids, which may be used as reference media in "*in vitro*" methods, such as common buffers or liquids. Such common buffers or liquids are known to a skilled person. Ringer-Lactate solution is particularly preferred as a liquid basis.

However, one or more compatible solid or liquid fillers or diluents or encapsulating compounds may be used as well for the inventive pharmaceutical composition, which are suitable for administration to a patient to be treated. The term "compatible" as used here means that these constituents of the inventive pharmaceutical composition are capable of being mixed with the at least one "complexed precomplexed nucleic acid" as defined according to the present invention in such a manner that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the inventive pharmaceutical composition under typical use conditions. Pharmaceutically acceptable carriers, fillers and diluents must, of course, have sufficiently high purity and sufficiently low toxicity to make them suitable for administration to a person to be treated. Some examples of compounds which can be used as pharmaceutically acceptable carriers, fillers or constituents thereof are sugars, such as, for example, lactose, glucose and sucrose; starches, such as, for example, corn starch or potato starch; cellulose and its derivatives, such as, for example, sodium carboxymethylcellulose, ethylcellulose, cellulose acetate; powdered tragacanth; malt; gelatin; tallow; solid glidants, such as, for example, stearic acid, magnesium stearate; calcium sulfate; vegetable oils, such as, for example, groundnut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil from theobroma; polyols, such as, for example, polypropylene glycol, glycerol, sorbitol, mannitol and polyethylene glycol; alginic acid.

According to another embodiment, the inventive pharmaceutical composition may comprise an adjuvant. In this context, an adjuvant may be understood as any compound, which is suitable to initiate or increase an immune response of the innate immune system, i.e. a non-specific immune response. With other words, when administered, the inventive pharmaceutical composition typically elicits an innate immune response due to the adjuvant, optionally contained therein. Such an adjuvant may be selected from any adjuvant known to a skilled person and suitable for the present case, i.e. supporting the induction of an innate immune response in a mammal, except of cationic or polycationic compounds as defined above in order to prevent complexation of the at least one free

mRNA. Preferably, the adjuvant may be selected from the group consisting of, without being limited thereto, including chitosan, TDM, MDP, muramyl dipeptide, pluronics, alum solution, aluminium hydroxide, ADJUMER™ (polyphosphazene); aluminium phosphate gel; glucans from algae; algammulin; aluminium hydroxide gel (alum); highly protein-adsorbing aluminium hydroxide gel; low viscosity aluminium hydroxide gel; AF or SPT (emulsion of squalane (5%), Tween 80 (0.2%), Pluronic L121 (1.25%), phosphate-buffered saline, pH 7.4); AVRIDINE™ (propanediamine); BAY R1005™ ((N-(2-deoxy-2-L-leucylamino-b-D-glucopyranosyl)-N-octadecyl-dodecanoyl-amide hydroacetate); CALCITRIOL™ (1-alpha,25-dihydroxy-vitamin D3); calcium phosphate gel; CAPTM (calcium phosphate nanoparticles); cholera holotoxin, cholera-toxin-A1-protein-A-D-fragment fusion protein, sub-unit B of the cholera toxin; CRL 1005 (block copolymer P1205); cytokine-containing liposomes; DDA (dimethyldioctadecylammonium bromide); DHEA (dehydroepiandrosterone); DMPC (dimyristoylphosphatidylcholine); DMPG (dimyristoylphosphatidylglycerol); DOC/alum complex (deoxycholic acid sodium salt); Freund's complete adjuvant; Freund's incomplete adjuvant; gamma inulin; Gerbu adjuvant (mixture of: i) N-acetylglucosaminyl-(P1-4)-N-acetylmuramyl-L-alanyl-D-glutamine (GMDP), ii) dimethyldioctadecylammonium chloride (DDA), iii) zinc-L-proline salt complex (ZnPro-8); GM-CSF); GMDP (N-acetylglucosaminyl-(b1-4)-N-acetylmuramyl-L-alanyl-D-isoglutamine); imiquimod (1-(2-methypropyl)-1H-imidazo[4,5-c]quinoline-4-amine); ImmTher™ (N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-glycerol dipalmitate); DRVs (immunoliposomes prepared from dehydration-rehydration vesicles); interferon-gamma; interleukin-1beta; interleukin-2; interleukin-7; interleukin-12; ISCOMS™; ISCOPREP 7.0.3. ™; liposomes; LOXORIBINE™ (7-allyl-8-oxoguanosine); LT oral adjuvant (*E.coli* labile enterotoxin-protoxin); microspheres and microparticles of any composition; MF59™; (squalene-water emulsion); MONTANIDE ISA 51™ (purified incomplete Freund's adjuvant); MONTANIDE ISA 720™ (metabolisable oil adjuvant); MPL™ (3-Q-desacyl-4'-monophosphoryl lipid A); MTP-PE and MTP-PE liposomes ((N-acetyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1,2-dipalmitoyl-sn-glycero-3-(hydroxyphosphoryloxy))-ethylamide, monosodium salt); MURAMETIDE™ (Nac-Mur-L-Ala-D-Gln-OCH_3); MURAPALMITINE™ and D-MURAPALMITINE™ (Nac-Mur-L-Thr-D-isoGln-sn-glyceroldipalmitoyl); NAGO (neuraminidase-galactose oxidase); nanospheres or nanoparticles of any composition; NISVs (non-ionic surfactant vesicles); PLEURAN™ (β-glucan); PLGA, PGA and PLA (homo- and co-polymers of lactic acid and glycolic acid; microspheres/nanospheres); PLURONIC L121™; PMMA (polymethyl methacrylate);

PODDS™ (proteinoid microspheres); polyethylene carbamate derivatives; poly-rA: poly-rU (polyadenylic acid-polyuridylic acid complex); polysorbate 80 (Tween 80); protein cochleates (Avanti Polar Lipids, Inc., Alabaster, AL); STIMULON™ (QS-21); Quil-A (Quil-A saponin); S-28463 (4-amino-otec-dimethyl-2-ethoxymethyl-1H-imidazo[4,5-c]quinoline-1-ethanol); SAF-1™ ("Syntex adjuvant formulation"); Sendai proteoliposomes and Sendai-containing lipid matrices; Span-85 (sorbitan trioleate); Specol (emulsion of Marcol 52, Span 85 and Tween 85); squalene or Robane® (2,6,10,15,19,23-hexamethyltetracosan and 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexane); stearyltyrosine (octadecyltyrosine hydrochloride); Theramid® (N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-dipalmitoxypropylamide); Theronyl-MDP (Termurtide™ or [thr 1]-MDP; N-acetylmuramyl-L-threonyl-D-isoglutamine); Ty particles (Ty-VLPs or virus-like particles); Walter-Reed liposomes (liposomes containing lipid A adsorbed on aluminium hydroxide), and lipopeptides, including Pam3Cys, in particular aluminium salts, such as Adju-phos, Alhydrogel, Rehydragel; emulsions, including CFA, SAF, IFA, MF59, Provax, TiterMax, Montanide, Vaxfectin; copolymers, including Optivax (CRL1005), L121, Poloaxmer4010), etc.; liposomes, including Stealth, cochleates, including BIORAL; plant derived adjuvants, including QS21, Quil A, Iscomatrix, ISCOM; adjuvants suitable for costimulation including Tomatine, biopolymers, including PLG, PMM, Inulin,; microbe derived adjuvants, including Romurtide, DETOX, MPL, CWS, Mannose, CpG nucleic acid sequences, CpG7909, ligands of human TLR 1-10, ligands of murine TLR 1-13, ISS-1018, IC31, Imidazoquinolines, Ampligen, Ribi529, IMOxine, IRIVs, VLPs, cholera toxin, heat-labile toxin, Pam3Cys, Flagellin, GPI anchor, LNFPIII/Lewis X, antimicrobial peptides, UC-1V150, RSV fusion protein, cdiGMP; and adjuvants suitable as antagonists including CGRP neuropeptide. Suitable adjuvants may furthermore be selected from lipid modified nucleic acids.

The inventive pharmaceutical composition may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or infusion techniques.

Preferably, the inventive pharmaceutical composition may be administered by parenteral injection, more preferably by subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or via infusion techniques. Sterile injectable forms of the inventive pharmaceutical compositions may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation of the inventive pharmaceutical composition.

The inventive pharmaceutical composition as defined above may also be administered orally in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient, i.e. the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

The inventive pharmaceutical composition may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, e.g. including diseases of the skin or of any other accessible epithelial tissue. Suitable topical formulations are readily prepared for each of these areas or organs. For topical applications, the inventive pharmaceutical composition may be formulated in a suitable ointment, containing the inventive immunostimulatory composition, particularly its components as defined above, suspended or dissolved in one or more carriers. Carriers for topical administration include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the inventive pharmaceutical composition can be formulated in a suitable lotion or cream. In the context of the present invention, suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The inventive pharmaceutical composition typically comprises a "safe and effective amount" of the components of the inventive pharmaceutical composition, particularly of the at least one "complexed precomplexed nucleic acid" as defined according to the present invention. As used herein, a "safe and effective amount" means an amount of the at least one "complexed precomplexed nucleic acid" as defined according to the present invention that is sufficient to significantly induce a positive modification of a disease or disorder as defined herein. At the same time, however, a "safe and effective amount" is small enough to avoid serious side-effects, that is to say to permit a sensible relationship between advantage and risk. The determination of these limits typically lies within the scope of sensible medical judgment. A "safe and effective amount" of the components of the inventive pharmaceutical composition, particularly of the the at least one "complexed precomplexed nucleic acid" as defined according to the present invention will furthermore vary in connection with the particular condition to be treated and also with the age and physical condition of the patient to be treated, the body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the activity of the specific "complexed precomplexed nucleic acid" as defined herein employed, the severity of the condition, the duration of the treatment, the nature of the accompanying therapy, of the particular pharmaceutically acceptable carrier used, and similar factors, within the knowledge and experience of the

accompanying doctor. The inventive pharmaceutical composition may be used for human and also for veterinary medical purposes, preferably for human medical purposes, as a pharmaceutical composition in general or as a vaccine.

According to a specific embodiment, the inventive pharmaceutical composition may be provided as a vaccine. Such an inventive vaccine is typically composed like the inventive pharmaceutical composition and preferably supports or elicits an immune response of the immune system of a patient to be treated, e.g. an innate immune response, if an RNA or mRNA is used as the nucleic acid molecule of the at least one "complexed precomplexed nucleic acid" as defined according to the present invention. Furthermore or alternatively, the inventive vaccine may elicit an adaptive immune response, preferably, if the the at least one "complexed precomplexed nucleic acid" as defined according to the present invention encodes any of the above mentioned antigens or proteins, which elicit an adaptive immune response.

The inventive vaccine may also comprise a pharmaceutically acceptable carrier, adjuvant, and/or vehicle as defined above for the inventive pharmaceutical composition. In the specific context of the inventive vaccine, the choice of a pharmaceutically acceptable carrier is determined in principle by the manner in which the inventive vaccine is administered. The inventive vaccine can be administered, for example, systemically or locally. Routes for systemic administration in general include, for example, transdermal, oral, parenteral routes, including subcutaneous, intravenous, intramuscular, intraarterial, intradermal and intraperitoneal injections and/or intranasal administration routes. Routes for local administration in general include, for example, topical administration routes but also intradermal, transdermal, subcutaneous, or intramuscular injections or intralesional, intracranial, intrapulmonal, intracardial, and sublingual injections. More preferably, vaccines may be administered by an intradermal, subcutaneous, or intramuscular route. Inventive vaccines are therefore preferably formulated in liquid (or sometimes in solid) form. The suitable amount of the inventive vaccine to be administered can be determined by routine experiments with animal models. Such models include, without implying any limitation, rabbit, sheep, mouse, rat, dog and non-human primate models. Preferred unit dose forms for injection include sterile solutions of water, physiological saline or mixtures thereof. The pH of such solutions should be adjusted to about 7.4. Suitable carriers for

injection include hydrogels, devices for controlled or delayed release, polylactic acid and collagen matrices. Suitable pharmaceutically acceptable carriers for topical application include those which are suitable for use in lotions, creams, gels and the like. If the inventive vaccine is to be administered orally, tablets, capsules and the like are the preferred unit dose form. The pharmaceutically acceptable carriers for the preparation of unit dose forms which can be used for oral administration are well known in the prior art. The choice thereof will depend on secondary considerations such as taste, costs and storability, which are not critical for the purposes of the present invention, and can be made without difficulty by a person skilled in the art.

The inventive vaccine can additionally contain one or more auxiliary substances in order to further increase its immunogenicity. A synergistic action of the at least one "complexed precomplexed nucleic acid" as defined according to the present invention and of an auxiliary substance, which may be optionally contained in the inventive vaccine as described above, is preferably achieved thereby. Depending on the various types of auxiliary substances, various mechanisms can come into consideration in this respect. For example, compounds that permit the maturation of dendritic cells (DCs), for example lipopolysaccharides, TNF-alpha or CD40 ligand, form a first class of suitable auxiliary substances. In general, it is possible to use as auxiliary substance any agent that influences the immune system in the manner of a "danger signal" (LPS, GP96, etc.) or cytokines, such as GM-CFS, which allow an immune response produced by the immune-stimulating adjuvant according to the invention to be enhanced and/or influenced in a targeted manner. Particularly preferred auxiliary substances are cytokines, such as monokines, lymphokines, interleukins or chemokines, that further promote the innate immune response, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, INF-alpha, IFN-beta, INF-gamma, GM-CSF, G-CSF, M-CSF, LT-beta or TNF-alpha, growth factors, such as hGH.

Further additives which may be included in the inventive vaccine are emulsifiers, such as, for example, Tween®; wetting agents, such as, for example, sodium lauryl sulfate; colouring agents; taste-imparting agents, pharmaceutical carriers; tablet-forming agents; stabilizers; antioxidants; preservatives.

The inventive vaccine can also additionally or alternatively contain any further compound, which is known to be immune-stimulating due to its binding affinity (as ligands) to human Toll-like receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, or due to its binding affinity (as ligands) to murine Toll-like receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12 or TLR13.

Another class of compounds, which may be added to an inventive vaccine in this context, may be CpG nucleic acids, in particular CpG-RNA or CpG-DNA. A CpG-RNA or CpG-DNA can be a single-stranded CpG-DNA (ss CpG-DNA), a double-stranded CpG-DNA (dsDNA), a single-stranded CpG-RNA (ss CpG-RNA) or a double-stranded CpG-RNA (ds CpG-RNA). The CpG nucleic acid is preferably in the form of CpG-RNA, more preferably in the form of single-stranded CpG-RNA (ss CpG-RNA). The CpG nucleic acid preferably contains at least one or more (mitogenic) cytosine/guanine dinucleotide sequence(s) (CpG motif(s)). According to a first preferred alternative, at least one CpG motif contained in these sequences, that is to say the C (cytosine) and the G (guanine) of the CpG motif, is unmethylated. All further cytosines or guanines optionally contained in these sequences can be either methylated or unmethylated. According to a further preferred alternative, however, the C (cytosine) and the G (guanine) of the CpG motif can also be present in methylated form.

The inventive vaccine can also additionally or alternatively contain an immunostimulatory RNA, i.e. an RNA derived from an immunostimulatory RNA, which triggers or increases an (innate) immune response. Preferably, such an RNA may be in general be as defined above for RNAs. In this context, those classes of RNA molecules, which can induce an innate immune response, may be selected e.g. from ligands of Toll-like receptors (TLRs), particularly from RNA sequences representing and/or encoding ligands of TLRs, preferably selected from human family members TLR1 – TLR10 or murine family members TLR1 – TLR13, more preferably from TLR7 and TLR8, ligands for intracellular receptors for RNA (such as RIG-I or MDA-5, etc.) (see e.g. Meylan, E., Tschopp, J. (2006). Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. Mol. Cell 22, 561-569), or any other immunostimulatory RNA sequence. Such an immunostimulatory RNA may

comprise a length of 1000 to 5000, of 500 to 5000, of 5 to 5000, or of 5 to 1000, 5 to 500, 5 to 250, of 5 to 100, of 5 to 50 or of 5 to 30 nucleotides.

According to a particularly preferred embodiment, the inventive vaccine may contain immunostimulatory RNA sequences of formula (I):

$$G_lX_mG_n \quad ,$$

wherein:

G is guanosine, uracil or an analogue of guanosine or uracil;

X is guanosine, uracil, adenosine, thymidine, cytosine or an analogue of the above-mentioned nucleotides;

l is an integer from 1 to 40,

wherein when l = 1 G is guanosine or an analogue thereof,

when l > 1 at least 50% of the nucleotides are guanosine or an analogue thereof;

m is an integer and is at least 3;

wherein when m = 3 X is uracil or an analogue thereof,

when m > 3 at least 3 successive uracils or analogues of uracil occur;

n is an integer from 1 to 40,

wherein when n = 1 G is guanosine or an analogue thereof,

when n > 1 at least 50% of the nucleotides are guanosine or an analogue thereof.

According to a another particularly preferred embodiment, the inventive vaccine may contain immunostimulatory RNA sequences of formula (II):

$$C_lX_mC_n \quad ,$$

wherein:

C is cytosine, uracil or an analogue of cytosine or uracil;

X is guanosine, uracil, adenosine, thymidine, cytosine or an analogue of the above-mentioned nucleotides;

l is an integer from 1 to 40,

wherein when l = 1 C is cytosine or an analogue thereof,

when l > 1 at least 50% of the nucleotides are cytosine or an analogue thereof;

m is an integer and is at least 3;

wherein when m = 3 X is uracil or an analogue thereof,

when m > 3 at least 3 successive uracils or analogues of uracil occur;

n is an integer from 1 to 40,

wherein when n = 1 C is cytosine or an analogue thereof,

when n > 1 at least 50% of the nucleotides are cytosine or an analogue thereof.

The nucleic acids of formula (I) or (II), which may be used for the inventive vaccine are typically relatively short nucleic acid molecules and typically have a length of approximately from 5 to 100 (but may also be longer than 100 nucleotides for specific embodiments, e.g. up to 200 nucleotides), from 5 to 90 or from 5 to 80 nucleotides, preferably a length of approximately from 5 to 70, more preferably a length of approximately from 8 to 60 and, more preferably a length of approximately from 15 to 60 nucleotides, more preferably from 20 to 60, most preferably from 30 to 60 nucleotides. If the nucleic acid of the invention has a maximum length of e.g. 100 nucleotides, m will typically be <=98. The number of nucleotides G in the nucleic acid of formula (I) is determined by l or n. l and n, independently of one another, are each an integer from 1 to 40, wherein when l or n = 1 G is guanosine or an analogue thereof, and when l or n > 1 at least 50% of the nucleotides are guanosine or an analogue thereof. For example, without implying any limitation, when l or n = 4 G_l or G_n can be, for example, a GUGU, GGUU, UGUG, UUGG, GUUG, GGGU, GGUG, GUGG, UGGG or GGGG, etc.; when l or n = 5 G_l or G_n can be, for example, a GGGUU, GGUGU, GUGGU, UGGGU, UGGUG, UGUGG, UUGGG, GUGUG, GGGGU, GGGUG, GGUGG, GUGGG, UGGGG, or GGGGG, etc.; etc. A nucleotide adjacent to X_m in the nucleic acid of formula (I) according to the invention is preferably not a uracil. Similarly, the number of nucleotides C in the nucleic acid of formula (II) according to the invention is determined by l or n. l and n, independently of one another, are each an integer from 1 to 40, wherein when l or n = 1 C is cytosine or an analogue thereof, and when l or n > 1 at least 50% of the nucleotides are cytosine or an analogue thereof. For example, without implying any limitation, when l or n = 4, C_l or C_n can be, for example, a CUCU, CCUU, UCUC, UUCC, CUUC, CCCU, CCUC, CUCC, UCCC or CCCC, etc.; when l or n = 5 C_l or C_n can be, for example, a CCCUU, CCUCU, CUCCU, UCCCU, UCCUC, UCUCC, UUCCC, CUCUC, CCCCU, CCCUC,

CCUCC, CUCCC, UCCCC, or CCCCC, etc.; etc. A nucleotide adjacent to X_m in the nucleic acid of formula (II) according to the invention is preferably not a uracil. Preferably, for formula (I), when l or n > 1, at least 60%, 70%, 80%, 90% or even 100% of the nucleotides are guanosine or an analogue thereof, as defined above. The remaining nucleotides to 100% (when guanosine constitutes less than 100% of the nucleotides) in the flanking sequences G_l and/or G_n are uracil or an analogue thereof, as defined hereinbefore. Also preferably, l and n, independently of one another, are each an integer from 2 to 30, more preferably an integer from 2 to 20 and yet more preferably an integer from 2 to 15. The lower limit of l or n can be varied if necessary and is at least 1, preferably at least 2, more preferably at least 3, 4, 5, 6, 7, 8, 9 or 10. This definition applies correspondingly to formula (II).

According to another particularly preferred embodiment, the inventive vaccine may contain immunostimulatory RNA sequences selected from a sequence consisting or comprising any of the following sequences:

GGUUUUUUUUUUUUUUGGG (SEQ ID NO: 10);

GGGGGUUUUUUUUUUGGGGG (SEQ ID NO: 11);

GGGGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGGGG (SEQ ID NO: 12);

GUGUGUGUGUGUUUUUUUUUUUUUUUGUGUGUGUGUGU (SEQ ID NO: 13);

GGUUGGUUGGUUUUUUUUUUUUUUUUGGUUGGUUGGUU (SEQ ID NO: 14);

GGGUUUUUUUUUUUUUUUGGGUUUUUUUUUUUUUUUGGGUUUUUUUUUUUU
UUUGGG (SEQ ID NO: 15);

GGGUUUUUUUUUUUUUUUGGGGGUUUUUUUUUUUUUUUGGG
(SEQ ID NO: 16),

CCCUUUUUUUUUUUUUUUCCCUUUUUUUUUUUUUUUCCCUUUUUUUUUUUU
UUCCC SEQ ID NO: 17,

CCCUUUCCCUUUCCCUUUCCCUUUCCCUUUCCCUUUCCCUUUCCCUUUCCC
SEQ ID NO: 18, or

CCCUUUUUUUUUUUUUUUCCCCCCUUUUUUUUUUUUUUUCCC
SEQ ID NO: 19,

or from a sequence having at least 60%, 70%, 80%, 90%, or even 95% sequence identity with any of these sequences

According to a particularly preferred embodiment, the inventive vaccine may contain immunostimulatory RNA sequences of formula (I):

According to a further preferred object of the present invention, the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, i.e. at least one complexed precomplexed nucleic acid (molecule), e.g. an mRNA, which has been precomplexed in a first step with (a high-molecular) PEI in an N/P ratio between 0.05 and 2, preferably in an N/P ratio between 0.1 and 1 and have been further complexed in a second step with a cationic or polycationic compound as defined above, may be used for the preparation of a pharmaceutical composition or a vaccine, preferably all as defined herein, for the prophylaxis, treatment and/or amelioration of any of the diseases and disorders as defined herein, e.g. cancer or tumor diseases, infectious diseases, preferably (viral, bacterial or protozoological) infectious diseases, autoimmune diseases, allergies or allergic diseases, monogenetic diseases, i.e. (hereditary) diseases, or genetic diseases in general, cardiovascular diseases and/or neural diseases.

Accordingly, the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, i.e. at least one complexed precomplexed nucleic acid (molecule), e.g. an mRNA, which has been precomplexed in a first step with (a high-molecular) PEI in an N/P ratio between 0.05 and 2, preferably in an N/P ratio between 0.1 and 1 and have been further complexed in a second step with a cationic or polycationic compound as defined above, may be used (for the preparation of a medicament, e.g. a pharmaceutical composition or a vaccine as defined above) for the prophylaxis, treatment and/or amelioration of e.g. cancer or tumor diseases, preferably selected from melanomas, malignant melanomas, colon carcinomas, lymphomas, sarcomas, blastomas, renal carcinomas, gastrointestinal tumors, gliomas, prostate tumors, bladder cancer, rectal tumors, stomach cancer, oesophageal cancer, pancreatic cancer, liver cancer, mammary carcinomas (= breast cancer), uterine cancer, cervical cancer, acute myeloid leukaemia (AML), acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), hepatomas, various virus-induced tumors such as, for example, papilloma virus-induced carcinomas (e.g. cervical carcinoma = cervical cancer),

adenocarcinomas, herpes virus-induced tumors (e.g. Burkitt's lymphoma, EBV-induced B-cell lymphoma), heptatitis B-induced tumors (hepatocell carcinomas), HTLV-1- and HTLV-2-induced lymphomas, acoustic neuroma, lung carcinomas (= lung cancer = bronchial carcinoma), small-cell lung carcinomas, pharyngeal cancer, anal carcinoma, glioblastoma, rectal carcinoma, astrocytoma, brain tumors, retinoblastoma, basalioma, brain metastases, medulloblastomas, vaginal cancer, pancreatic cancer, testicular cancer, Hodgkin's syndrome, meningiomas, Schneeberger disease, hypophysis tumor, Mycosis fungoides, carcinoids, neurinoma, spinalioma, Burkitt's lymphoma, laryngeal cancer, renal cancer, thymoma, corpus carcinoma, bone cancer, non-Hodgkin's lymphomas, urethral cancer, CUP syndrome, head/neck tumors, oligodendroglioma, vulval cancer, intestinal cancer, colon carcinoma, oesophageal carcinoma (= oesophageal cancer), wart involvement, tumors of the small intestine, craniopharyngeomas, ovarian carcinoma, genital tumors, ovarian cancer (= ovarian carcinoma), pancreatic carcinoma (= pancreatic cancer), endometrial carcinoma, liver metastases, penile cancer, tongue cancer, gall bladder cancer, leukaemia, plasmocytoma, lid tumor, prostate cancer (= prostate tumors), etc.

Furthermore, the at least one "complexed precomplexed nucleic acid" as defined according to the present invention may be used (for the preparation of a medicament, e.g. a pharmaceutical composition or a vaccine as defined above) for the prophylaxis, treatment, and/or amelioration of e.g. infectious diseases, preferably (viral, bacterial or protozoological) infectious diseases. Such infectious diseases, preferably to (viral, bacterial or protozoological) infectious diseases, are typically selected from influenza, malaria, SARS, yellow fever, AIDS, Lyme borreliosis, Leishmaniasis, anthrax, meningitis, viral infectious diseases such as AIDS, Condyloma acuminata, hollow warts, Dengue fever, three-day fever, Ebola virus, cold, early summer meningoencephalitis (FSME), flu, shingles, hepatitis, herpes simplex type I, herpes simplex type II, Herpes zoster, influenza, Japanese encephalitis, Lassa fever, Marburg virus, measles, foot-and-mouth disease, mononucleosis, mumps, Norwalk virus infection, Pfeiffer's glandular fever, smallpox, polio (childhood lameness), pseudo-croup, fifth disease, rabies, warts, West Nile fever, chickenpox, cytomegalic virus (CMV), bacterial infectious diseases such as miscarriage (prostate inflammation), anthrax, appendicitis, borreliosis, botulism, Camphylobacter, Chlamydia trachomatis (inflammation of the urethra, conjunctivitis), cholera, diphtheria, donavanosis, epiglottitis, typhus fever, gas gangrene, gonorrhoea, rabbit fever, Heliobacter pylori, whooping cough, climatic bubo,

osteomyelitis, Legionnaire's disease, leprosy, listeriosis, pneumonia, meningitis, bacterial meningitis, anthrax, otitis media, Mycoplasma hominis, neonatal sepsis (Chorioamnionitis), noma, paratyphus, plague, Reiter's syndrome, Rocky Mountain spotted fever, Salmonella paratyphus, Salmonella typhus, scarlet fever, syphilis, tetanus, tripper, tsutsugamushi disease, tuberculosis, typhus, vaginitis (colpitis), soft chancre, and infectious diseases caused by parasites, protozoa or fungi, such as amoebiasis, bilharziosis, Chagas disease, Echinococcus, fish tapeworm, fish poisoning (Ciguatera), fox tapeworm, athlete's foot, canine tapeworm, candidosis, yeast fungus spots, scabies, cutaneous Leishmaniosis, lambliasis (giardiasis), lice, malaria, microscopy, onchocercosis (river blindness), fungal diseases, bovine tapeworm, schistosomiasis, porcine tapeworm, toxoplasmosis, trichomoniasis, trypanosomiasis (sleeping sickness), visceral Leishmaniosis, nappy/diaper dermatitis or miniature tapeworm.

Likewise, the at least one "complexed precomplexed nucleic acid" as defined according to the present invention may be used (for the preparation of a medicament, e.g. a pharmaceutical composition or a vaccine as defined above) for the prophylaxis, treatment, and/or amelioration of e.g. autoimmune diseases. Autoimmune diseases can be broadly divided into systemic and organ-specific or localised autoimmune disorders, depending on the principal clinico-pathologic features of each disease. Autoimmune diseases may be divided into the categories of systemic syndromes, including systemic lupus erythematosus (SLE), Sjögren's syndrome, Scleroderma, Rheumatoid Arthritis and polymyositis or local syndromes which may be endocrinologic (type I diabetes (Diabetes mellitus Type 1), Hashimoto's thyroiditis, Addison's disease etc.), dermatologic (pemphigus vulgaris), haematologic (autoimmune haemolytic anaemia), neural (multiple sclerosis) or can involve virtually any circumscribed mass of body tissue. The autoimmune diseases to be treated may be selected from the group consisting of type I autoimmune diseases or type II autoimmune diseases or type III autoimmune diseases or type IV autoimmune diseases, such as, for example, multiple sclerosis (MS), rheumatoid arthritis, diabetes, type I diabetes (Diabetes mellitus Type 1), chronic polyarthritis, Basedow's disease, autoimmune forms of chronic hepatitis, colitis ulcerosa, type I allergy diseases, type II allergy diseases, type III allergy diseases, type IV allergy diseases, fibromyalgia, hair loss, Bechterew's disease, Crohn's disease, Myasthenia gravis, neurodermitis, Polymyalgia rheumatica, progressive systemic sclerosis (PSS), Reiter's syndrome, rheumatic arthritis, psoriasis, vasculitis, etc, or

type II diabetes. While the exact mode as to why the immune system induces a immune reaction against autoantigens has not been elucidated so far, there are several findings with regard to the etiology. Accordingly, the autoreaction may be due to a T-Cell bypass. A normal immune system requires the activation of B-cells by T-cells before the former can produce antibodies in large quantities. This requirement of a T-cell can be by-passed in rare instances, such as infection by organisms producing super-antigens, which are capable of initiating polyclonal activation of B-cells, or even of T-cells, by directly binding to the ß-subunit of T-cell receptors in a non-specific fashion. Another explanation deduces autoimmune diseases from a Molecular Mimicry. An exogenous antigen may share structural similarities with certain host antigens; thus, any antibody produced against this antigen (which mimics the self-antigens) can also, in theory, bind to the host antigens and amplify the immune response. The most striking form of molecular mimicry is observed in Group A beta-haemolytic streptococci, which shares antigens with human myocardium, and is responsible for the cardiac manifestations of rheumatic fever.

Furthermore, the at least one "complexed precomplexed nucleic acid" as defined according to the present invention may be used (for the preparation of a medicament, e.g. a pharmaceutical composition or a vaccine as defined above) for the prophylaxis, treatment, and/or amelioration of allergies or allergic diseases, i.e. diseases related to allergies. Allergy is a condition that typically involves an abnormal, acquired immunological hypersensitivity to certain foreign antigens or allergens, such as the allergy antigens as defined above. Such allergy antigens or allergens may be selected from allergy antigens as defined above antigens derived from different sources, e.g. from animals, plants, fungi, bacteria, etc. Allergens in this context include e.g. grasses, pollens, molds, drugs, or numerous environmental triggers, etc. Allergies normally result in a local or systemic inflammatory response to these antigens or allergens and lead to immunity in the body against these allergens. Without being bound to theory, several different disease mechanisms are supposed to be involved in the development of allergies. According to a classification scheme by P. Gell and R. Coombs the word "allergy" was restricted to type I hypersensitivities, which are caused by the classical IgE mechanism. Type I hypersensitivity is characterised by excessive activation of mast cells and basophils by IgE, resulting in a systemic inflammatory response that can result in symptoms as benign as a runny nose, to life-threatening anaphylactic shock and death. Well known types of allergies include,

without being limited thereto, allergic asthma (leading to swelling of the nasal mucosa), allergic conjunctivitis (leading to redness and itching of the conjunctiva), allergic rhinitis ("hay fever"), anaphylaxis, angiodema, atopic dermatitis (eczema), urticaria (hives), eosinophilia, respiratory, allergies to insect stings, skin allergies (leading to and including various rashes, such as eczema, hives (urticaria) and (contact) dermatitis), food allergies, allergies to medicine, etc. With regard to the present invention, the inventive immunostimulatory composition, the inventive pharmaceutical composition or the inventive vaccine, or the at least one (m)RNA of the adjuvant component of the inventive immunostimulatory composition complexed with a cationic or polycationic compound, together with the at least one free mRNA of the inventive immunostimulatory composition, may be used for the treatment of such allergic disorders or diseases, preferably by desensitizing the immune reaction which triggers a specific immune response. Such a desensitizing may be carried out by administering an effective amount of the allergen or allergic antigen encoded by a RNA of the inventive immunostimulatory composition, preferably the at least one free mRNA, to induce a slight immune reaction. The amount of the allergen or allergic antigen may then be raised step by step in subsequent administrations until the immune system of the patient to be treated tolerates a specific amount of allergen or allergic antigen.

Additionally, diseases to be treated in the context of the present invention likewise include (hereditary) diseases, or genetic diseases in general monogenetic diseases, i.e. (hereditary) diseases, or genetic diseases in general. Accordingly, the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, may be used (for the preparation of a medicament, e.g. a pharmaceutical composition or a vaccine as defined above) for the prophylaxis, treatment, and/or amelioration of genetic diseases. Such (mono-)genetic diseases, (hereditary) diseases, or genetic diseases in general are typically caused by genetic defects, e.g. due to gene mutations resulting in loss of protein activity or regulatory mutations which do not allow transcription or translation of the protein. Frequently, these diseases lead to metabolic disorders or other symptoms, e.g. muscle dystrophy. Accordingly, the present invention allows to treat these diseases by providing the complexed RNA as defined herein. Insofar, the following diseases may be treated: 3-beta-hydroxysteroid dehydrogenase deficiency (type II); 3-ketothiolase deficiency; 6-mercaptopurine sensitivity; Aarskog-Scott syndrome; Abetalipoproteinemia; Acatalasemia;

Achondrogenesis; Achondrogenesis-hypochondrogenesis; Achondroplasia; Achromatopsia; Acromesomelic dysplasia (Hunter-Thompson type); ACTH deficiency; Acyl-CoA dehydrogenase deficiency (short-chain, medium chain, long chain); Adenomatous polyposis coli; Adenosin-deaminase deficiency; Adenylosuccinase deficiency; Adhalinopathy; Adrenal hyperplasia, congenital (due to 11-beta-hydroxylase deficiency; due to 17-alpha-hydroxylase deficiency; due to 21-hydroxylase deficiency); Adrenal hypoplasia, congenital, with hypogonadotropic hypogonadism; Adrenogenital syndrom; Adrenoleukodystrophy; Adrenomyeloneuropathy; Afibrinogenemia; Agammaglobulinemia; Alagille syndrome; Albinism (brown, ocular, oculocutaneous, rufous); Alcohol intolerance, acute; Aldolase A deficiency; Aldosteronism, glucocorticoid-remediable; Alexander disease; Alkaptonuria; Alopecia universalis; Alpha-1-antichymotrypsin deficiency; Alpha-methylacyl-CoA racemase deficiency; Alpha-thalassemia/mental retardation syndrome; Alport syndrome; Alzheimer disease-1 (APP-related); Alzheimer disease-3; Alzheimer disease-4; Amelogenesis imperfecta; Amyloid neuropathy (familial, several allelic types); Amyloidosis (Dutch type; Finnish type; hereditary renal; renal; senile systemic); Amytrophic lateral sclerosis; Analbuminemia; Androgen insensitivity; Anemia (Diamond-Blackfan); Anemia (hemolytic, due to PK deficiency); Anemia (hemolytic, Rh-null, suppressor type); Anemia (neonatal hemolytic, fatal and nearfatal); Anemia (sideroblastic, with ataxia); Anemia (sideroblastic/hypochromic); Anemia due to G6PD deficiency; Aneurysm (familial arterial); Angelman syndrome; Angioedema; Aniridia; Anterior segment anomalies and cataract; Anterior segment mesenchymal dysgenesis; Anterior segment mesenchymal dysgenesis and cataract; Antithrombin III deficiency; Anxiety-related personality traits; Apert syndrome; Apnea (postanesthetic); ApoA-I and apoC-III deficiency (combined); Apolipoprotein A-II deficiency; Apolipoprotein B-100 (ligand-defective); Apparent mineralocorticoid excess (hypertension due to); Argininemia; Argininosuccinicaciduria; Arthropathy (progressive pseudorheumatoid, of childhood); Aspartylglucosaminuria; Ataxia (episodic); Ataxia with isolated vitamin E deficiency; Ataxia-telangiectasia; Atelosteogenesis II; ATP-dependent DNA ligase I deficiency; Atrial septal defect with atrioventricular conduction defects; Atrichia with papular lesions; Autism (succinylpurinemic); Autoimmune polyglandular disease, type I; Autonomic nervous system dysfunction; Axenfeld anomaly; Azoospermia; Bamforth-Lazarus syndrome; Bannayan-Zonana syndrome; Barthsyndrome; Bartter syndrome (type 2 or type 3); Basal cell carcinoma ; Basal cell nevus syndrome; BCG infection; Beare-Stevenson cutis gyrata syndrome; Becker muscular dystrophy; Beckwith-

Wiedemann syndrome; Bernard-Soulier syndrome (type B; type C); Bethlem myopathy; Bile acid malabsorption, primary ; Biotinidase deficiency; Bladder cancer; Bleeding disorder due to defective thromboxane A2 receptor; Bloom syndrome; Brachydactyly (type B1 or type C); Branchiootic syndrome; Branchiootorenal syndrome; Breast cancer (invasive intraductal; lobular; male, with Reifenstein syndrome; sporadic); Breast cancer-1 (early onset); Breast cancer-2 (early onset); Brody myopathy; Brugada syndrome; Brunner syndrome; Burkitt lymphoma; Butterfly dystrophy (retinal); C1q deficiency (type A; type B; type C); C1r/C1s deficiency; C1s deficiency, isolated; C2 deficiency ; C3 deficiency; C3b inactivator deficiency; C4 deficiency; C8 deficiency, type II; C9 deficiency; Campomelic dysplasia with autosomal sex reversal; Camptodactyly-arthropathy-coxa varapericarditis syndrome; Canavan disease; Carbamoylphosphate synthetase I deficiency; Carbohydrate-deficient glycoprotein syndrome (type I; type Ib; type II); Carcinoid tumor of lung; Cardioencephalomyopathy (fatal infantile, due to cytochrome c oxidase deficiency); Cardiomyopathy (dilated; X-linked dilated; familial hypertrophic; hypertrophic); Carnitine deficiency (systemic primary); Carnitine-acylcarnitine translocase deficiency; Carpal tunnel syndrome (familial); Cataract (cerulean; congenital; crystalline aculeiform; juvenile-onset; polymorphic and lamellar; punctate; zonular pulverulent); Cataract, Coppock-like; CD59 deficiency; Central core disease; Cerebellar ataxia; Cerebral amyloid angiopathy; Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy; Cerebral cavernous malformations-1; Cerebrooculofacioskeletal syndrome; Cerebrotendinous xanthomatosis; Cerebrovascular disease; Ceroid lipofuscinosis (neuronal, variant juvenile type, with granular osmiophilic deposits); Ceroid lipofuscinosis (neuronal-1, infantile); Ceroid-lipofuscinosis (neuronal-3, juvenile); Char syndrome; Charcot-Marie-Tooth disease; Charcot-Marie-Tooth neuropathy; Charlevoix-Saguenay type; Chediak-Higashi syndrome; Chloride diarrhea (Finnish type); Cholestasis (benign recurrent intrahepatic); Cholestasis (familial intrahepatic); Cholestasis (progressive familial intrahepatic); Cholesteryl ester storage disease; Chondrodysplasia punctata (brachytelephalangic; rhizomelic; X-linked dominant; X-linked recessive; Grebe type); Chondrosarcoma; Choroideremia; Chronic granulomatous disease (autosomal, due to deficiency of CYBA); Chronic granulomatous disease (X-linked); Chronic granulomatous disease due to deficiency of NCF-1; Chronic granulomatous disease due to deficiency of NCF-2; Chylomicronemia syndrome, familial; Citrullinemia; classical Cockayne syndrome-1; Cleft lip, cleft jaw, cleft palate; Cleft lip/palate ectodermal dysplasia syndrome; Cleidocranial dysplasia; CMO II deficiency;

Coats disease; Cockayne syndrome-2, type B; Coffin-Lowry syndrome; Colchicine resistance; Colon adenocarcinoma; Colon cancer; Colorblindness (deutan; protan; tritan); Colorectal cancer; Combined factor V and VIII deficiency; Combined hyperlipemia (familial); Combined immunodeficiency (X-linked, moderate); Complex I deficiency; Complex neurologic disorder; Cone dystrophy-3; Cone-rod dystrophy 3; Cone-rod dystrophy 6; Cone-rod retinal dystrophy-2; Congenital bilateral absence of vas deferens; Conjunctivitis, ligneous; Contractural arachnodactyly; Coproporphyria; Cornea plana congenita; Corneal clouding; Corneal dystrophy (Avellino type; gelatinous drop-like; Groenouw type I; lattice type I; Reis-Bucklers type); Cortisol resistance; Coumarin resistance; Cowden disease; CPT deficiency, hepatic (type I; type II); Cramps (familial, potassium-aggravated); Craniofacial-deafness-hand syndrome; Craniosynostosis (type 2); Cretinism; Creutzfeldt-Jakob disease ; Crigler-Najjar syndrome; Crouzon syndrome; Currarino syndrome; Cutis laxa; Cyclic hematopoiesis; Cyclic ichthyosis; Cylindromatosis; Cystic fibrosis; Cystinosis (nephropathic); Cystinuria (type II; type III); Daltonism; Darier disease; D-bifunctional protein deficiency; Deafness, autosomal dominant 1; Deafness, autosomal dominant 11; Deafness, autosomal dominant 12; Deafness, autosomal dominant 15; Deafness, autosomal dominant 2; Deafness, autosomal dominant 3; Deafness, autosomal dominant 5; Deafness, autosomal dominant 8; Deafness, autosomal dominant 9; Deafness, autosomal recessive 1; Deafness, autosomal recessive 2; Deafness, autosomal recessive 21; Deafness, autosomal recessive 3; Deafness, autosomal recessive 4; Deafness, autosomal recessive 9; Deafness, nonsyndromic sensorineural 13; Deafness, X-linked 1; Deafness, X-linked 3; Debrisoquine sensitivity; Dejerine-Sottas disease; Dementia (familial Danish); Dementia (frontotemporal, with parkinsonism); Dent disease; Dental anomalies; Dentatorubro-pallidoluysian atrophy; Denys-Drash syndrome; Dermatofibrosarcoma protuberans; Desmoid disease; Diabetes insipidus (nephrogenic); Diabetes insipidus (neurohypophyseal); Diabetes mellitus (insulin-resistant); Diabetes mellitus (rare form); Diabetes mellitus (type II); Diastrophic dysplasia; Dihydropyrimidinuria; Dosage-sensitive sex reversal; Doyne honeycomb degeneration of retina; Dubin-Johnson syndrome; Duchenne muscular dystrophy; Dyserythropoietic anemia with thrombocytopenia; Dysfibrinogenemia (alpha type; beta type; gamma type); Dyskeratosis congenita-1; Dysprothrombinemia; Dystonia (DOPAresponsive); Dystonia (myoclonic); Dystonia-1 (torsion); Ectodermal dysplasia; Ectopia lentis; Ectopia pupillae; Ectrodactyly (ectodermal dysplasia, and cleft lip/palate syndrome 3); Ehlers-Danlos syndrome (progeroid form);

Ehlers-Danlos syndrome (type I; type II; type III; type IV; type VI; type VII); Elastin Supravalvar aortic stenosis; Elliptocytosis-1; Elliptocytosis-2; Elliptocytosis-3; Ellis-van Creveld syndrome; Emery-Dreifuss muscular dystrophy; Emphysema; Encephalopathy; Endocardial fibroelastosis-2; Endometrial carcinoma; Endplate acetylcholinesterase deficiency; Enhanced S-cone syndrome; Enlarged vestibular aqueduct; Epidermolysis bullosa; Epidermolysis bullosa dystrophica (dominant or recessive); Epidermolysis bullosa simplex; Epidermolytic hyperkeratosis; Epidermolytic palmoplantar keratoderma; Epilepsy (generalize; juvenile; myoclonic; nocturnal frontal lobe; progressive myoclonic); Epilepsy, benign, neonatal (type1 or type2); Epiphyseal dysplasia (multiple); Episodic ataxia (type 2); Episodic ataxia/myokymia syndrome; Erythremias (alpha-; dysplasia); Erythrocytosis; Erythrokeratoderma; Estrogen resistance; Exertional myoglobinuria due to deficiency of LDH-A; Exostoses, multiple (type 1; type 2); Exudative vitreoretinopathy, X-linked; Fabry disease; Factor H deficiency; Factor VII deficiency; Factor X deficiency; Factor XI deficiency; Factor XII deficiency; Factor XIIIA deficiency; Factor XIIIB deficiency; Familial Mediterranean fever; Fanconi anemia; Fanconi-Bickel syndrome; Farber lipogranulomatosis; Fatty liver (acute); Favism; Fish-eye disease; Foveal hypoplasia; Fragile X syndrome; Frasier syndrome; Friedreich ataxia; fructose-bisphosphatase Fructose intolerance; Fucosidosis; Fumarase deficiency; Fundus albipunctatus; Fundus flavimaculatus; G6PD deficiency; GABA-transaminase deficiency; Galactokinase deficiency with cataracts; Galactose epimerase deficiency; Galactosemia; Galactosialidosis; GAMT deficiency; Gardner syndrome; Gastric cancer; Gaucher disease; Generalized epilepsy with febrile seizures plus; Germ cell tumors; Gerstmann-Straussler disease; Giant cell hepatitis (neonatal); Giant platelet disorder; Giant-cell fibroblastoma; Gitelman syndrome; Glanzmann thrombasthenia (type A; type B); Glaucoma 1A; Glaucoma 3A; Glioblastoma multiforme; Glomerulosclerosis (focal segmental); Glucose transport defect (blood-brain barrier); Glucose/galactose malabsorption; Glucosidase I deficiency; Glutaricaciduria (type I; type IIB; type IIC); Gluthation synthetase deficiency; Glycerol kinase deficiency; Glycine receptor (alpha-1 polypeptide); Glycogen storage disease I; Glycogen storage disease II; Glycogen storage disease III; Glycogen storage disease IV; Glycogen storage disease VI; Glycogen storage disease VII; Glycogenosis (hepatic, autosomal); Glycogenosis (X-linked hepatic); GM1-gangliosidosis; GM2-gangliosidosis; Goiter (adolescent multinodular); Goiter (congenital); Goiter (nonendemic, simple); Gonadal dysgenesis (XY type); Granulomatosis, septic; Graves disease; Greig cephalopolysyndactyly syndrome; Griscelli syndrome; Growth

hormone deficient dwarfism; Growth retardation with deafness and mental retardation; Gynecomastia (familial, due to increased aromatase activity); Gyrate atrophy of choroid and retina with ornithinemia (B6 responsive or unresponsive); Hailey-Hailey disease; Haim-Munk syndrome; Hand-foot-uterus syndrome; Harderoporphyrinuria; HDL deficiency (familial); Heart block (nonprogressive or progressive); Heinz body anemia; HELLP syndrome; Hematuria (familial benign); Heme oxygenase-1 deficiency; Hemiplegic migraine; Hemochromotosis; Hemoglobin H disease; Hemolytic anemia due to ADA excess; Hemolytic anemia due to adenylate kinase deficiency; Hemolytic anemia due to band 3 defect; Hemolytic anemia due to glucosephosphate isomerase deficiency; Hemolytic anemia due to glutathione synthetase deficiency; Hemolytic anemia due to hexokinase deficiency; Hemolytic anemia due to PGK deficiency; Hemolytic-uremic syndrome; Hemophagocytic lymphohistiocytosis; Hemophilia A; Hemophilia B; Hemorrhagic diathesis due to factor V deficiency; Hemosiderosis (systemic, due to aceruloplasminemia); Hepatic lipase deficiency; Hepatoblastoma; Hepatocellular carcinoma; Hereditary hemorrhagic telangiectasia-1; Hereditary hemorrhagic telangiectasia-2; Hermansky-Pudlak syndrome; Heterotaxy (X-linked visceral); Heterotopia (periventricular); Hippel-Lindau syndrom; Hirschsprung disease; Histidine-rich glycoprotein Thrombophilia due to HRG deficiency; HMG-CoA lyase deficiency; Holoprosencephaly-2; Holoprosencephaly-3; Holoprosencephaly-4; Holoprosencephaly-5; Holt-Oram syndrome; Homocystinuria; Hoyeraal-Hreidarsson; HPFH (deletion type or nondeletion type); HPRT-related gout; Huntington disease; Hydrocephalus due to aqueductal stenosis; Hydrops fetalis; Hyperbetalipoproteinemia; Hypercholesterolemia, familial; Hyperferritinemia-cataract syndrome; Hyperglycerolemia; Hyperglycinemia; Hyperimmunoglobulinemia D and periodic fever syndrome; Hyperinsulinism; Hyperinsulinism-hyperammonemia syndrome; Hyperkalemic periodic paralysis; Hyperlipoproteinemia; Hyperlysinemia; Hypermethioninemia (persistent, autosomal, dominant, due to methionine, adenosyltransferase I/III deficiency); Hyperornithinemia-hyperammonemiahomocitrullinemia syndrome; Hyperoxaluria; Hyperparathyroidism; Hyperphenylalaninemia due to pterin-4acarbinolamine dehydratase deficiency; Hyperproinsulinemia; Hyperprolinemia; Hypertension; Hyperthroidism (congenital); Hypertriglyceridemia; Hypoalphalipoproteinemia; Hypobetalipoproteinemia; Hypocalcemia; Hypochondroplasia; Hypochromic microcytic anemia; Hypodontia; Hypofibrinogenemia; Hypoglobulinemia and absent B cells; Hypogonadism

(hypergonadotropic); Hypogonadotropic (hypogonadism); Hypokalemic periodic paralysis; Hypomagnesemia; Hypomyelination (congenital); Hypoparathyroidism; Hypophosphatasia (adult; childhood; infantile; hereditary); Hypoprothrombinemia; Hypothyroidism (congenital; hereditary congenital; nongoitrous); Ichthyosiform erythroderma ; Ichthyosis ; Ichthyosis bullosa of Siemens ; IgG2 deficiency; Immotile cilia syndrome-1; Immunodeficiency (T-cell receptor/CD3 complex); Immunodeficiency (X-linked, with hyper-IgM); Immunodeficiency due to defect in CD3-gamma; Immunodeficiency-centromeric instabilityfacial anomalies syndrome; Incontinentia pigmenti; Insensitivity to pain (congenital, with anhidrosis); Insomnia (fatal familial); Interleukin-2 receptor deficiency (alpha chain); Intervertebral disc disease; Iridogoniodysgenesis; Isolated growth hormone deficiency (Illig type with absent GH and Kowarski type with bioinactive GH); Isovalericacidemia ; Jackson-Weiss sydnrome; Jensen syndrome; Jervell and Lange-Nielsen syndrome; Joubert syndrom; Juberg-Marsidi syndrome; Kallmann syndrome; Kanzaki disease; Keratitis; Keratoderma (palmoplantar); Keratosis palmoplantaris striata I; Keratosis palmoplantaris striata II; Ketoacidosis due to SCOT deficiency; Keutel syndrome; Klippel-Trenaurnay syndrom; Kniest dysplasia; Kostmann neutropenia; Krabbe disease; Kurzripp-Polydaktylie syndrom; Lacticacidemia due to PDX1 deficiency; Langer mesomelic dysplasia; Laron dwarfism; Laurence-Moon-Biedl-Bardet syndrom; LCHAD deficiency; Leber congenital amaurosis; Left-right axis malformation; Leigh syndrome; Leiomyomatosis (diffuse, with Alport syndrome); Leprechaunism; Leri- Weill dyschondrosteosis; Lesch-Nyhan syndrome; Leukemia (acute myeloid; acute promyelocytic; acute T-cell lymphoblastic; chronic myeloid; juvenile myelomonocytic; Leukemia-1 (T-cell acute lymphocytic); Leukocyte adhesion deficiency; Leydig cell adenoma; Lhermitte-Duclos syndrome; Liddle syndrome; Li-Fraumeni syndrome; Lipoamide dehydrogenase deficiency; Lipodystrophy; Lipoid adrenal hyperplasia; Lipoprotein lipase deficiency; Lissencephaly (X-linked); Lissencephaly-1; liver Glycogen storage disease (type 0); Long QT syndrome-1; Long QT syndrome-2; Long QT syndrome-3; Long QT syndrome-5; Long QT syndrome-6; Lowe syndrome; Lung cancer; Lung cancer (nonsmall cell); Lung cancer (small cell); Lymphedema; Lymphoma (B-cell non-Hodgkin); Lymphoma (diffuse large cell); Lymphoma (follicular); Lymphoma (MALT); Lymphoma (mantel cell); Lymphoproliferative syndrome (X-linked); Lysinuric protein intolerance; Machado-Joseph disease; Macrocytic anemia refractory (of 5q syndrome); Macular dystrophy; Malignant mesothelioma; Malonyl-CoA decarboxylase deficiency ; Mannosidosis, (alpha- or beta-); Maple syrup urine disease (type

Ia; type Ib; type II); Marfan syndrome; Maroteaux-Lamy syndrome; Marshall syndrome; MASA syndrome; Mast cell leukemia; Mastocytosis with associated hematologic disorder; McArdle disease; McCune-Albright polyostotic fibrous dysplasia; McKusick-Kaufman syndrome; McLeod phenotype ; Medullary thyroid carcinoma; Medulloblastoma; Meesmann corneal dystrophy; Megaloblastic anemia-1; Melanoma; Membroproliferative glomerulonephritis ; Meniere disease; Meningioma (NF2-related; SIS-related); Menkes disease; Mental retardation (X-linked); Mephenytoin poor metabolizer; Mesothelioma; Metachromatic leukodystrophy; Metaphyseal chondrodysplasia (Murk Jansen type; Schmid type); Methemoglobinemia; Methionine adenosyltransferase deficiency (autosomal recessive); Methylcobalamin deficiency (cbl G type); Methylmalonicaciduria (mutase deficiency type); Mevalonicaciduria; MHC class II deficiency; Microphthalmia (cataracts, and iris abnormalities); Miyoshi myopathy; MODY; Mohr-Tranebjaerg syndrome; Molybdenum cofactor deficiency (type A or type B); Monilethrix; Morbus Fabry; Morbus Gaucher; Mucopolysaccharidosis; Mucoviscidosis; Muencke syndrome; Muir-Torre syndrome; Mulibrey nanism; Multiple carboxylase deficiency (biotinresponsive); Multiple endocrine neoplasia; Muscle glycogenosis; Muscular dystrophy (congenital merosindeficient); Muscular dystrophy (Fukuyama congenital); Muscular dystrophy (limb-girdle); Muscular dystrophy) Duchenne-like); Muscular dystrophy with epidermolysis bullosa simplex; Myasthenic syndrome (slow-channel congenital); Mycobacterial infection (atypical, familial disseminated); Myelodysplastic syndrome; Myelogenous leukemia; Myeloid malignancy; Myeloperoxidase deficiency; Myoadenylate deaminase deficiency; Myoglobinuria/hemolysis due to PGK deficiency; Myoneurogastrointestinal encephalomyopathy syndrome; Myopathy (actin; congenital; desmin-related; cardioskeletal; distal; nemaline); Myopathy due to CPT II deficiency; Myopathy due to phosphoglycerate mutase deficiency; Myotonia congenita; Myotonia levior; Myotonic dystrophy; Myxoid liposarcoma; NAGA deficiency; Nailpatella syndrome; Nemaline myopathy 1 (autosomal dominant); Nemaline myopathy 2 (autosomal recessive); Neonatal hyperparathyroidism; Nephrolithiasis; Nephronophthisis (juvenile); Nephropathy (chronic hypocomplementemic); Nephrosis-1; Nephrotic syndrome; Netherton syndrome; Neuroblastoma; Neurofibromatosis (type 1 or type 2); Neurolemmomatosis; neuronal-5 Ceroid-lipofuscinosis; Neuropathy; Neutropenia (alloimmune neonatal); Niemann-Pick disease (type A; type B; type C1; type D); Night blindness (congenital stationary); Nijmegen breakage syndrome; Noncompaction of left ventricular myocardium; Nonepidermolytic palmoplantar keratoderma; Norrie

disease; Norum disease; Nucleoside phosphorylase deficiency; Obesity; Occipital hornsyndrome; Ocular albinism (Nettleship-Falls type); Oculopharyngeal muscular dystorphy; Oguchi disease; Oligodontia; Omenn syndrome; Opitz G syndrome; Optic nerve coloboma with renal disease; Ornithine transcarbamylase deficiency; Oroticaciduria; Orthostatic intolerance; OSMED syndrome; Ossification of posterior longitudinal ligament of spine; Osteoarthrosis; Osteogenesis imperfecta; Osteolysis; Osteopetrosis (recessive or idiopathic); Osteosarcoma; Ovarian carcinoma; Ovarian dysgenesis; Pachyonychia congenita (Jackson-Lawler type or Jadassohn-Lewandowsky type); Paget disease of bone; Pallister-Hall syndrome; Pancreatic agenesis; Pancreatic cancer; Pancreatitis; Papillon-Lefevre syndrome; Paragangliomas ; Paramyotonia congenita; Parietal foramina; Parkinson disease (familial or juvenile); Paroxysmal nocturnal hemoglobinuria; Pelizaeus-Merzbacher disease; Pendred syndrome; Perineal hypospadias; Periodic fever; Peroxisomal biogenesis disorder; Persistent hyperinsulinemic hypoglycemia of infancy; Persistent Mullerian duct syndrome (type II); Peters anomaly; Peutz-Jeghers syndrome ; Pfeiffer syndrome; Phenylketonuria; Phosphoribosyl pyrophosphate synthetaserelated gout; Phosphorylase kinase deficiency of liver and muscle; Piebaldism; Pilomatricoma; Pinealoma with bilateral retinoblastoma; Pituitary ACTH secreting adenoma; Pituitary hormone deficiency; Pituitary tumor; Placental steroid sulfatase deficiency; Plasmin inhibitor deficiency; Plasminogen deficiency (types I and II); Plasminogen Tochigi disease; Platelet disorder; Platelet glycoprotein IV deficiency; Platelet-activating factor acetylhydrolase deficiency; Polycystic kidney disease; Polycystic lipomembranous osteodysplasia with sclerosing leukenencephalophathy; Polydactyly, postaxial; Polyposis; Popliteal pterygium syndrome; Porphyria (acute hepatic or acute intermittent or congenital erythropoietic); Porphyria cutanea tarda; Porphyria hepatoerythropoietic ; Porphyria variegata; Prader-Willi syndrome; Precocious puberty; Premature ovarian failure; Progeria Typ I; Progeria Typ II; Progressive external ophthalmoplegia; Progressive intrahepatic cholestasis-2; Prolactinoma (hyperparathyroidism, carcinoid syndrome); Prolidase deficiency; Propionicacidemia; Prostate cancer; Protein S deficiency; Proteinuria; Protoporphyria (erythropoietic); Pseudoachondroplasia; Pseudohermaphroditism; Pseudohypoaldosteronism; Pseudohypoparathyroidism; Pseudovaginal perineoscrotal hypospadias; Pseudovitamin D deficiency rickets; Pseudoxanthoma elasticum (autosomal dominant; autosomal recessive); Pulmonary alveolar proteinosis; Pulmonary hypertension; Purpura fulminans; Pycnodysostosis; Pyropoikilocytosis; Pyruvate carboxylase deficiency; Pyruvate

dehydrogenase deficiency; Rabson-Mendenhall syndrome; Refsum disease; Renal cell carcinoma; Renal tubular acidosis; Renal tubular acidosis with deafness; Renal tubular acidosis-osteopetrosis syndrome; Reticulosis (familial histiocytic); Retinal degeneration; Retinal dystrophy; Retinitis pigmentosa; Retinitis punctata albescens; Retinoblastoma; Retinol binding protein deficiency; Retinoschisis; Rett syndrome; Rh(mod) syndrome; Rhabdoid predisposition syndrome; Rhabdoid tumors ; Rhabdomyosarcoma; Rhabdomyosarcoma (alveolar); Rhizomelic chondrodysplasia punctata; Ribbing-Syndrom; Rickets (vitamin D-resistant); Rieger anomaly; Robinow syndrome; Rothmund-Thomson syndrome; Rubenstein-Taybi syndrome; Saccharopinuria; Saethre-Chotzen syndrome; Salla disease; Sandhoff disease (infantile, juvenile, and adult forms); Sanfilippo syndrome (type A or type B); Schindler disease; Schizencephaly; Schizophrenia (chronic); Schwannoma (sporadic); SCID (autosomal recessive, T-negative/Bpositive type); Secretory pathway w/TMD; SED congenita; Segawa syndrome; Selective T-cell defect; SEMD (Pakistani type); SEMD (Strudwick type); Septooptic dysplasia; Severe combined immunodeficiency (B cellnegative); Severe combined immunodeficiency (T-cell negative, B-cell/natural killer cell-positive type); Severe combined immunodeficiency (Xlinked); Severe combined immunodeficiency due to ADA deficiency; Sex reversal (XY, with adrenal failure); Sezary syndrome; Shah-Waardenburg syndrome; Short stature; Shprintzen-Goldberg syndrome; Sialic acid storage disorder; Sialidosis (type I or type II); Sialuria; Sickle cell anemia; Simpson-Golabi-Behmel syndrome; Situs ambiguus; Sjogren-Larsson syndrome; Smith-Fineman-Myers syndrome; Smith-Lemli-Opitz syndrome (type I or type II); Somatotrophinoma; Sorsby fundus dystrophy; Spastic paraplegia; Spherocytosis; Spherocytosis-1; Spherocytosis-2; Spinal and bulbar muscular atrophy of Kennedy; Spinal muscular atrophy; Spinocerebellar ataxia; Spondylocostal dysostosis; Spondyloepiphyseal dysplasia tarda; Spondylometaphyseal dysplasia (Japanese type); Stargardt disease-1; Steatocystoma multiplex; Stickler syndrome; Sturge-Weber syndrom; Subcortical laminal heteropia; Subcortical laminar heterotopia; Succinic semialdehyde dehydrogenase deficiency; Sucrose intolerance; Sutherland-Haan syndrome; Sweat chloride elevation without CF; Symphalangism; Synostoses syndrome; Synpolydactyly; Tangier disease; Tay-Sachs disease; T-cell acute lymphoblastic leukemia; T-cell immunodeficiency; T-cell prolymphocytic leukemia; Thalassemia (alpha- or delta-); Thalassemia due to Hb Lepore; Thanatophoric dysplasia (types I or II); Thiamine-responsive megaloblastic anemia syndrome; Thrombocythemia; Thrombophilia (dysplasminogenemic); Thrombophilia due to

heparin cofactor II deficiency; Thrombophilia due to protein C deficiency; Thrombophilia due to thrombomodulin defect; Thyroid adenoma; Thyroid hormone resistance; Thyroid iodine peroxidase deficiency; Tietz syndrome; Tolbutamide poor metabolizer; Townes-Brocks syndrome; Transcobalamin II deficiency; Treacher Collins mandibulofacial dysostosis; Trichodontoosseous syndrome; Trichorhinophalangeal syndrome; Trichothiodystrophy; Trifunctional protein deficiency (type I or type II); Trypsinogen deficiency; Tuberous sclerosis-1; Tuberous sclerosis-2; Turcot syndrome; Tyrosine phosphatase; Tyrosinemia; Ulnar-mammary syndrome; Urolithiasis (2,8-dihydroxyadenine); Usher syndrome (type 1B or type 2A); Venous malformations; Ventricular tachycardia; Virilization; Vitamin K-dependent coagulation defect; VLCAD deficiency; Vohwinkel syndrome; von Hippel-Lindau syndrome; von Willebrand disease; Waardenburg syndrome; Waardenburg syndrome/ocular albinism; Waardenburg-Shah neurologic variant; Waardenburg-Shah syndrome; Wagner syndrome; Warfarin sensitivity; Watson syndrome; Weissenbacher-Zweymuller syndrome; Werner syndrome; Weyers acrodental dysostosis; White sponge nevus; Williams-Beuren syndrome; Wilms tumor (type1); Wilson disease; Wiskott-Aldrich syndrome; Wolcott-Rallison syndrome; Wolfram syndrome; Wolman disease; Xanthinuria (type I); Xeroderma pigmentosum; X-SCID; Yemenite deaf-blind hypopigmentation syndrome; ypocalciuric hypercalcemia (type I); Zellweger syndrome; Zlotogora-Ogur syndrome.

Preferred diseases to be treated which have a genetic inherited background and which are typically caused by a single gene defect and are inherited according to Mendel's laws are preferably selected from the group consisting of autosomal-recessive inherited diseases, such as, for example, adenosine deaminase deficiency, familial hypercholesterolaemia, Canavan's syndrome, Gaucher's disease, Fanconi anaemia, neuronal ceroid lipofuscinoses, mucoviscidosis (cystic fibrosis), sickle cell anaemia, phenylketonuria, alcaptonuria, albinism, hypothyreosis, galactosaemia, alpha-1-anti-trypsin deficiency, Xeroderma pigmentosum, Ribbing's syndrome, mucopolysaccharidoses, cleft lip, jaw, palate, Laurence Moon Biedl Bardet sydrome, short rib polydactylia syndrome, cretinism, Joubert's syndrome, type II progeria, brachydactylia, adrenogenital syndrome, and X-chromosome inherited diseases, such as, for example, colour blindness, e.g. red/green blindness, fragile X syndrome, muscular dystrophy (Duchenne and Becker-Kiener type), haemophilia A and B, G6PD deficiency, Fabry's disease, mucopolysaccharidosis, Norrie's syndrome, Retinitis

pigmentosa, septic granulomatosis, X-SCID, ornithine transcarbamylase deficiency, Lesch-Nyhan syndrome, or from autosomal-dominant inherited diseases, such as, for example, hereditary angiooedema, Marfan syndrome, neurofibromatosis, type I progeria, Osteogenesis imperfecta, Klippel-Trenaurnay syndrome, Sturge-Weber syndrome, Hippel-Lindau syndrome and tuberosis sclerosis.

The present invention also allows treatment of diseases, which have not been inherited, or which may not be summarized under the above categories. Such dieseases may include e.g. the treatment of patients, which are in need of a specific protein factor, e.g. a specific therapeutically active protein as mentioned above. This may e.g. include dialysis patients, e.g. patients which undergo a (regular) a kidney or renal dialysis, and which may be in need of specific therapeutically active proteins as defined above, e.g. erythropoietin (EPO), etc.

Likewise, the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, may be used for (the preparation of a medicament, e.g. a pharmaceutical composition or a vaccine as defined above for) the prophylaxis, treatment, and/or amelioration of cardiovascular diseases chosen from, without being limited thereto, coronary heart disease, arteriosclerosis, apoplexy and hypertension, etc.

Finally, the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, may be used for (the preparation of a medicament, e.g. a pharmaceutical composition or a vaccine as defined above for) the prophylaxis, treatment, and/or amelioration of neuronal diseases chosen from Alzheimer's disease, amyotrophic lateral sclerosis, dystonia, epilepsy, multiple sclerosis and Parkinson's disease etc.

In the context of the above, the invention furthermore relates also to the use of the inventive pharmaceutical composition or the inventive vaccine, or the components thereof, i.e. the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, preferably the at least one complexed precomplexed nucleic acid (molecule), e.g. an mRNA, which has been precomplexed in a first step with (a high-molecular) PEI in an N/P ratio between 0.05 and 2, preferably in an N/P ratio between 0.1 and 1 and have been further complexed in a second step with a cationic or polycationic compound as defined above, for the prophylaxis, treatment, and/or amelioration of diseases or disorders

as mentioned herein. It also includes in particular the use of the inventive pharmaceutical composition or the inventive vaccine, or the components thereof as an inoculant. According to one particularly preferred embodiment of the present invention, such a method for prophylaxis, treatment, and/or amelioration of the above-mentioned diseases or disorders, or an inoculation method for preventing the above-mentioned diseases, typically comprises administering the described pharmaceutical composition or vaccine or components thereof to a patient in need thereof (e.g. suffering from any of the above diseases or showing symptoms thereof), in particular to a human being, preferably in a "safe and effective amount" and in one of the above formulations as described above for inventive pharmaceutical compositions. The administration mode also may be as described above for inventive pharmaceutical compositions or vaccines.

The present patent application also provides a method of transfecting and optionally administering the inventive pharmaceutical composition or the inventive vaccine, or its components as defined above, e.g. the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, comprising the following steps:

(a) collection of blood cells, professional antigen presenting cells (APCs), especially dendritic cells (DCs), and

(b) transfection of the blood cells, professional antigen presenting cells (APCs), especially dendritic cells (DCs), *in vitro* with inventive pharmaceutical composition or the inventive vaccine, or its components as defined above, e.g. the at least one "complexed precomplexed nucleic acid" as defined according to the present invention.

In the context of the present invention, "blood cells" are preferably understood according to the invention as meaning a mixture or an enriched to substantially pure population of red blood cells, granulocytes, mononuclear cells (PBMCs) and/or blood platelets from whole blood, blood serum or another source, e.g. from the spleen or lymph nodes, only a small proportion of professional APCs being present. Preferably, blood cells in the present invention are typically characterized in that they contain a small proportion of well-differentiated professional antigen presenting cells (APCs), especially dendritic cells (DCs). The blood cells as used according to the present invention may be preferably fresh blood cells, i.e. the period between collection of the blood cells (especially blood withdrawal) and

transfection being only short, e.g. less than 12 h, preferably less than 6 h, particularly preferably less than 2 h and very particularly preferably less than 1 h. However, the blood cells as used according to the present invention may also be blood cells obtained upon withdrawal of blood prior to need, e.g. an operation or a treatment as defined herein, and which are stored subsequently until use.

Blood cells may be collected from an animal or human patient by standard methods, for example. Thus whole blood can easily be obtained by puncturing a suitable vessel. Serum is obtained in known manner by coagulating the solid blood constituents. PBMCs may be mentioned as an example of an enriched partial population of blood cells. These are conventionally isolated by a method first described by Bøyum (Nature 204, 793-794, 1964; Scan. J. Lab. Clin. Invest. Suppl. 97, 1967). This is generally done by withdrawing blood from the individual and adding it e.g. to a solution of density 1.077 g/ml (25°C), conventionally containing Ficoll and sodium diatrizoate, for density gradient centrifugation. During careful centrifugation at room temperature, the PBMCs collect at the Ficoll/blood interface whereas the red blood cells and the remaining white blood cells are sedimented. The interface with the PBMCs is recovered and conventionally washed with a suitable buffer, e.g. sterile PBS. The PBMCs are preferably subjected to a short isotonic treatment with an aqueous solution of e.g. ammonium chloride. Finally, the PBMCs are washed a further one or more times with a buffer such as PBS (sterile). The cells obtained can then optionally be stored under suitable conditions, conventionally at -70°C, until further use.

According to one preferred embodiment, the blood cells immediately prior to transfection are fresh blood cells, i.e. there is only a short period between the collection of blood cells (especially blood withdrawal) in step (a) and the transfection according to step (b), e.g. less than 12 h, preferably less than 6 h, particularly preferably less than 2 h and very particularly preferably less than 1 h.

In the context of the present invention, dendritic cells (DCs), as used in the above mentioned inventive method of transfection and optionally administration, are typically potent antigen presenting cells (APCs) that typically possess the ability to stimulate naïve T cells. They comprise a system of leukocytes widely distributed in all tissues, especially in those that provide an environmental interface. DCs posses a heterogeneous haemopoietic

lineage, in that subsets from different tissues have been shown to posses a differential morphology, phenotype and function. The ability to stimulate naïve T cell proliferation appears to be shared between these various DC subsets. It has been suggested that the so-called myeloid and lymphoid-derived subsets of DCs perform specific stimulatory or tolerogenic function, respectively. DCs are derived from bone marrow progenitors and circulate in the blood as immature precursors prior to migration into peripheral tissues. Within different tissues, DCs differentiate and become active in the taking up and processing of antigens, and their subsequent presentation on the cell surface linked to major histocompatibility (MHC) molecules. Upon appropriate stimulation, DCs undergo further maturation and migrate to secondary lymphoid tissues where they present antigens to T cells and induce an immune response. DCs are receiving increasing scientific and clinical interest due to their key role in anti-cancer host responses and potential use as biological adjuvants in tumour vaccines, as well as their involvement in the immunobiology of tolerance and autoimmunity. Dendritic cells (DCs), however, may also be used in the context of the present invention, if no or a lower immune response is required or envisaged. Such dendritic cells (DCs), as defined above, may be isolated from different tissues as mentioned above or from blood, particularly from blood samples as described above.

Preferably, the blood cells, professional antigen presenting cells (APCs), especially dendritic cells (DCs), used for the inventive method of transfection and optionally administration of the inventive pharmaceutical composition or the inventive vaccine preferably originate from the actual patient who will be treated with the pharmaceutical composition of the present invention (autologous cells). The pharmaceutical composition or the vaccine according to the invention therefore preferably contains autologous blood cells, professional antigen presenting cells (APCs), especially dendritic cells (DCs). All types of autologous cells are encompassed hereby.

The transfection of the blood cells, professional antigen presenting cells (APCs), especially dendritic cells (DCs), is likewise carried out by common methods, e.g. by means of electroporation or chemical methods, especially lipofection, preferably only by administration of the inventive composition without further transfection reagents.

The the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, i.e. at least one complexed precomplexed nucleic acid (molecule), e.g. an mRNA, which has been precomplexed in a first step with (a high-molecular) PEI in an N/P ratio between 0.05 and 2, preferably in an N/P ratio between 0.1 and 1 and have been further complexed in a second step with a cationic or polycationic compound as defined above, is preferably prepared by methods known to those skilled in the art, especially by chemical synthesis or particularly preferably by means of molecular biological methods which have already been mentioned above, more preferably by methods as described above

As defined above, there may be the need to transfect the blood cells, professional antigen presenting cells (APCs), especially dendritic cells (DCs), used for the inventive method of transfection and optionally administration, with the inventive pharmaceutical composition or vaccine or its components, e.g. the at least one "complexed precomplexed nucleic acid" as defined according to the present invention. Furthermore, administration of such transfected cells to a patient, to living tissues and/or organisms *in vivo*, particularly retransplantation into the host organism in the case of autologous cells, may be envisaged as well and carried out as an optional step c) of the above mentioned method. In this context, an organism (or a being) typically means mammals, selected from, without being restricted thereto, the group comprising humans, and animals, including e.g. pig, goat, cattle, swine, dog, cat, donkey, monkey, ape or rodents, including mouse, hamster and rabbit. Furthermore, living tissues as mentioned above, are preferably derived from these organisms. Administration of the transfected cells to those living tissues and/or organisms may occur via any suitable administration route, e.g. systemically, and include e.g. intra- or transdermal, oral, parenteral, including subcutaneous, intramuscular or intravenous injections, topical and/or intranasal routes as defined above.

According to another embodiment, the present invention also provides a method for the preparation of the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, comprising following steps:

a) providing a nucleic acid precomplexed with PEI by precomplexing a nucleic acid (molecule) as defined above with PEI in a ratio as defined above, preferably in an N/P ratio between 0.05 and 2, more preferably in an N/P

ratio between 0.05 and 1, even more preferably in an N/P ratio between 0.1 and 1; and subsequently

b) complexing the nucleic acid precomplexed with PEI with a cationic or polycationic compound, preferably a cationic peptide, preferbly in an N/P ratio of about about 0.1-50, preferably in a range of about 0.5-50 and most preferably in a range of about 0.75-25 or 1-25 regarding the ratio of RNA:cationic peptide in the complex, even more preferably in the range of about 10-50 and most preferably in the range of about 25-50).

According to a final embodiment, the present invention also provides kits, particularly kits of parts, comprising as components alone or in combination, the inventive inventive pharmaceutical composition or vaccine, and/or the at least one "complexed precomplexed nucleic acid" as defined according to the present invention, and optionally technical instructions with information on the administration and dosage of these components. Such kits, preferably kits of parts, may be applied, e.g., for any of the above mentioned applications or uses.

Figures

The following Figures are intended to illustrate the invention further. They are not intended to limit the subject matter of the invention thereto.

Figure 1: shows a GC enriched luciferase encoding mRNA sequence according to SEQ ID NO: 1, which exhibits a length of 1863 nuclotides and was termed "pCV19-Pp luc(GC)-muag-A70-C30". The mRNA sequence of pCV19-Pp luc(GC)-muag-A70-C30 encodes luciferase and contains following sequence elements:

- GC-optimized sequence for a better codon usage and stabilization;
- stabilizing sequences derived from alpha-globin-3'-UTR (muag (mutated alpha-globin-3'-UTR));
- 70 × adenosine at the 3'-terminal end (poly-A-tail);
- 30 × cytosine at the 3'- terminal end (poly-C-tail).

Figure 2: shows a wildtype luciferase encoding mRNA sequence according to SEQ ID NO: 2, which exhibits a length of 1882 nuclotides and was termed "CAP-Ppluc(wt)-muag-A70-C30". The mRNA sequence of CAP-Ppluc(wt)-muag-A70-C30 encodes wildtype luciferase and contains following sequence elements:

- stabilizing sequences derived from alpha-globin-3'-UTR (muag (mutated alpha-globin-3'-UTR));
- 70 × adenosine at the 3'-terminal end (poly-A-tail);
- 30 × cytosine at the 3'- terminal end (poly-C-tail).

The ORF is indicated in italic letters, muag (mutated alpha-globin-3'-UTR is indicated with a dotted line, the poly-A-tail is underlined with a single line and the poly-C-tail is underlined with a double line.

Figure 3: shows a GC enriched luciferase encoding mRNA sequence according to SEQ ID NO: 3, which exhibits a length of 1857 nuclotides and was termed "CAP-Ppluc(GC)-muag-A70-C30". The mRNA sequence of CAP-Ppluc(wt)-muag-A70-C30 encodes luciferase and contains following sequence elements:

- GC-optimized sequence for a better codon usage and stabilization;
- stabilizing sequences derived from alpha-globin-3'-UTR (muag (mutated alpha-globin-3'-UTR));
- 70 × adenosine at the 3'-terminal end (poly-A-tail);
- 30 × cytosine at the 3'- terminal end (poly-C-tail).

The ORF is indicated in italic letters, muag (mutated alpha-globin-3'-UTR is indicated with a dotted line, the poly-A-tail is underlined with a single line and the poly-C-tail is underlined with a double line.

Figure 4: shows the coding sequence of CAP-Ppluc(wt)-muag-A70-C30 (SEQ ID NO: 2), which was assigned as SEQ ID NO: 4.

Figure 5: depicts the coding sequence of CAP-Ppluc(GC)-muag-A70-C30 (SEQ ID NO: 3), which was assigned as SEQ ID NO: 5.

Figure 6: shows that precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.67 and further complexed with the cationic peptide R_9H_3 (Figure 6, N/P PEI (RNA:peptide 1:5000) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone (Figure 6, N/P PEI w/o peptide) has no influence on luciferase expression (see Figure 6). The control was carried out with RNA alone (4 µg RNA) or without RNA (w/o RNA).

Figure 7: reflects that precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.67 and further complexed with the cationic peptide R_9H_3 (Figure 7, N/P PEI (molar ratio of RNA:peptide 1:5000, corresponding to an N/P ratio of about 25)) leads to a significantly increased luciferase expression in CHO-cells. The control was carried out with RNA alone (4 µg RNA), Peptide alone without RNA (w/o RNA) and medium without RNA (w/o RNA).

Figure 8: shows that precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.17 to 0.67 and further complexed with the cationic peptide R_9H_3 (Figure 8, N/P PEI (molar ratio of RNA:peptide 1:5000, corresponding to an N/P ratio of about 25)) leads to a significantly increased luciferase expression in L929-cells, whereas a complexation with PEI alone (Figure 8, N/P PEI w/o peptide) has no influence on luciferase expression (see Figure 8). The control was carried out with RNA alone (4 µg RNA).

Figure 9: shows that precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.17 and further complexed with the cationic peptide protamin in a mass ratio of 8:1 (corresponding to a molar ratio of 1:35 and an N/P ratio of 0.12) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone (Figure 9, w/o peptide) has no influence on luciferase expression.

Figure 10: depicts that precomplexation of the luciferase encoding mRNA, which has been precomplexed either high-molecular PEI (800 kDa) in an N/P ratio of 0.04 to 0.67 or low-molecular PEI (25 kDa) in an N/P ratio of 0.04, respectively, and further complexed with the cationic peptide R_9H_3 (Figure 10, N/P PEI 800 kDa (molar ratio of RNA:peptide 1:5000, corresponding to an N/P ratio of about 25) or N/P PEI 25 kDa (molar ratio of RNA:peptide 1:5000, corresponding to an N/P ratio of about 25)) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone (Figure 10, N/P PEI w/o peptide) has no influence on luciferase expression (see Figure 10). The control was carried out with RNA alone (4 µg RNA), with medium without RNA (w/o RNA) or with peptide without RNA (w/o RNA).

Figure 11: illustrates that precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.67 and further complexed with the cationic peptide R_9 (Figure 11, N/P PEI (molar

ratio of RNA:peptide 1:5000, corresponding to an N/P ratio of about 25)) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone (Figure 11, N/P PEI w/o) has no influence on luciferase expression (see Figure 11). The control was carried out with RNA alone (luc-RNActive 4 µg RNA) or with medium without RNA (medium w/o RNA).

Figure 12: shows that precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.17 and further complexed with the cationic polymer Lipofectamine (not Lipofectamine 2000) in an RNA:Lipofectamine ratio of 1µg:1µl (see Figure 12) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone has no influence on luciferase expression (see Figure 12). The control was carried out with RNA alone (luc-RNActive 4 µg RNA) or with medium without RNA (w/o RNA).

Examples

The following examples are intended to illustrate the invention further. They are not intended to limit the subject matter of the invention thereto.

1. Preparation of DNA and mRNA constructs encoding Pp luciferase (*Photinus pyralis*)

For the present examples DNA sequences, encoding Pp luciferase (*Photinus pyralis*), and corresponding mRNA sequences encoding Pp luciferase sequences, were prepared and used for subsequent *in vitro* transcription reactions, transfection and vaccination experiments.

According to a first preparation, the DNA sequence and corresponding mRNA sequences termed pCV19-Ppluc(GC)-muag-A70-C30 sequence were prepared, which corresponds to the luciferase coding sequence. The constructs were prepared by modifying the wildtype luciferase encoding DNA sequence by introducing a GC-optimized sequence for a better codon usage and stabilization, stabilizing sequences derived from alpha-globin-3'-UTR (muag (mutated alpha-globin-3'-UTR)), a stretch of 70 × adenosine at the 3'-terminal end (poly-A-tail) and a stretch of 30 × cytosine at the 3'- terminal end (poly-C-tail), leading to SEQ ID NO: 1 (see Figure 1). The sequence of the final DNA construct had a length of 1863 nucleotides and was termed "pCV19-Ppluc(GC)-muag-A70-C30". In SEQ ID NO: 1 (see Figure 1) the sequence of the corresponding mRNA is shown.

According to a second preparation, the DNA sequence and corresponding mRNA sequence termed CAP-Ppluc(GC)-muag-A70-C30 DNA sequence were prepared, which also correspond to the luciferase coding sequence. Therefore, a basic DNA construct was prepared termed CAP-Ppluc(wt)-muag-A70-C30 by introducing into the underlying wildtype sequence construct stabilizing sequences derived from alpha-globin-3'-UTR (muag (mutated alpha-globin-3'-UTR)), a stretch of 70 × adenosine at the 3'-terminal end (poly-A-tail) and a stretch of 30 × cytosine at the 3'-terminal end (poly-C-tail), leading to a sequence according to SEQ ID NO: 2 (see Figure 2). SEQ ID NO: 2 (see Figure 2) shows the corresponding mRNA sequence of

the entire wt construct. The mRNA coding sequence (CDS) of the basic construct termed CAP-Ppluc(wt)-muag-A70-C30 is shown in Figure 4 (SEQ ID NO: 4). This construct was further modified by introducing a GC-optimized sequence for a better codon usage and stabilization, leading to SEQ ID NO: 3 (see Figure 3). SEQ ID NO: 3 (see Figure 3) shows the corresponding mRNA sequence of the GC modified construct. The final GC modified construct had a length of 1857 nucleotides and was termed "CAP-Ppluc(GC)-muag-A70-C30". The mRNA coding sequence (CDS) of the final construct termed CAP-Ppluc(GC)-muag-A70-C30 is shown in Figure 5 (SEQ ID NO: 5).

2. *In vitro* transcription of stabilized luciferase mRNA constructs

The stabilized luciferase mRNA according to SEQ ID NOs: 1 or 3 were transcribed *in vitro* using T7-Polymerase (T7-Opti mRNA Kit, CureVac, Tübingen, Deutschland) starting from the above DNA constructs following the manufactures instructions.

All mRNA-transkripts contained a GC-optimized sequence for a better codon usage and stabilization, stabilizing sequences derived from alpha-globin-3'-UTR (muag (mutated alpha-globin-3'-UTR)), a stretch of 70 × adenosine at the 3'-terminal end (poly-A-tail), a stretch of 30 × cytosine at the 3'- terminal end (poly-C-tail), and a 5'-Cap-structure. The 5'-Cap-structure was obtained by adding an excess of N7-Methyl-Guanosin-5'-Triphosphat-5'-Guanosin.

3. Precomplexation and complexation of stabilized luciferase mRNA constructs

4 µg RNA stabilized luciferase mRNA according to SEQ ID NOs: 1 or 2 were precomplexed by mixing the mRNA in different N/P ratios with PEI (high molecular PEI (800 kDa) or low molecular PEI (25 kDa)) as specifically indicated in the following examples (precomplexed mRNA). Subsequently the mRNA-PEI precomplexes were further complexed by mixing the precomplexes with a cationic transfection reagent in the indicated molar ratio and diluted with water to a final volume of 40 µl, leading to "complexed precomplexed mRNA". The cationic transfection reagent used in the specific example (R_9H_3, R_9, protamine or lipofectamine (not lipofectamine 2000)) is indicated for each example in the following.

4. Transfection and expression of stabilized luciferase mRNA constructs

4.1 Hela-cells

For transfection with HeLa-cells, HeLa-cells ($1x10^5$/well) were seeded 1 day prior to transfection on 24-well microtiter plates leading to a 70% confluence when transfection was carried out. For transfection 40 µl of an RNA-solution containing a "precomplexed complexed mRNA" construct as disclosed above, e.g. a precomplexed complexed mRNA according to SEQ ID NO: 1 or 3, were mixed with 260 µl serum free medium and added to the cells (final RNA concentration of each preparation was 13 µg/ml). Prior to addition of the transfection solution the HeLa-cells were washed gently and carefully 2 times with 2 ml Optimen (Invitrogen) per well. Then, the transfection solution (300 µl per well) was added to the cells and the cells were incubated for 4 h at 37°C. Subsequently 300 µl RPMI-medium (Camprex) containing 10% FCS was added per well and the cells were incubated for additional 20 h at 37°C. The transfection solution was sucked off 24 h after transfection and the cells were lysed in 300 µl lysis buffer (25 mM Tris-PO_4, 2 mM EDTA, 10% glycerol, 1% Triton-X 100, 2 mM DTT). The supernatants were then mixed with luciferin buffer (25 mM Glycylglycin, 15 mM $MgSO_4$, 5 mM ATP, 62,5 µM luciferin) and luminiscence was detected using a luminometer (Lumat LB 9507 (Berthold Technologies, Bad Wildbad, Germany)).

4.2. CHO cells

For transfection with CHO-cells, CHO-cells ($1x10^5$/well) were seeded 1 day prior to transfection on 24-well microtiter plates leading to a 70% confluence when transfection was carried out. For transfection 40 µl of an RNA-solution containing a "precomplexed complexed mRNA" construct as disclosed above, e.g. a precomplexed complexed mRNA according to SEQ ID NO: 1 or 3, were mixed with 260 µl serum free medium and added to the cells (final RNA concentration of each preparation was 13 µg/ml). Prior to addition of the transfection solution the CHO-cells were washed gently and carefully 2 times with 2 ml Optimen (Invitrogen) per well. Then, the transfection solution (300 µl per well) was added to the cells and the cells were incubated for 4 h at 37°C. Subsequently 300 µl Ham's F12-medium was added per well and the cells were incubated for additional 20 h at 37°C. The

transfection solution was sucked off 24 h after transfection and the cells were lysed in 300 µl lysis buffer (25 mM Tris-PO_4, 2 mM EDTA, 10% glycerol, 1% Triton-X 100, 2 mM DTT). The supernatants were then mixed with luciferin buffer (25 mM Glycylglycin, 15 mM $MgSO_4$, 5 mM ATP, 62,5 µM luciferin) and luminiscence was detected using a luminometer (Lumat LB 9507 (Berthold Technologies, Bad Wildbad, Germany)).

4.3 L929 cells

For transfection with L929-cells, L929-cells ($1x10^5$/well) were seeded 1 day prior to transfection on 24-well microtiter plates leading to a 70% confluence when transfection was carried out. For transfection 40 µl of an RNA-solution containing a "precomplexed complexed mRNA" construct as disclosed above, e.g. a precomplexed complexed mRNA according to SEQ ID NO: 1 or 3, were mixed with 260 µl serum free medium and added to the cells (final RNA concentration of each preparation was 13 µg/ml). Prior to addition of the transfection solution the L929-cells were washed gently and carefully 2 times with 2 ml Optimen (Invitrogen) per well. Then, the transfection solution (300 µl per well) was added to the cells and the cells were incubated for 4 h at 37°C. Subsequently 300 µl RPMI-medium (Camprex) containing 10% FCS was added per well and the cells were incubated for additional 20 h at 37°C. The transfection solution was sucked off 24 h after transfection and the cells were lysed in 300 µl lysis buffer (25 mM Tris-PO_4, 2 mM EDTA, 10% glycerol, 1% Triton-X 100, 2 mM DTT). The supernatants were then mixed with luciferin buffer (25 mM Glycylglycin, 15 mM $MgSO_4$, 5 mM ATP, 62,5 µM luciferin) and luminiscence was detected using a luminometer (Lumat LB 9507 (Berthold Technologies, Bad Wildbad, Germany)).

4.4 Transfection of stabilized luciferase mRNA constructs in HeLa cells with high molecular PEI (800 kDa) and R_9H_3 as a cationic polymer

Preparing the precomplexed complexed mRNA constructs and the transfection into HeLa-cells was generally carried out as described above under sections 3. and 4.1. Particularly, HeLa-cells were transfected with a precomplexed complexed mRNA according to SEQ ID NO: 3 (mRNA concentration: 4 µg CAP-Ppluc(GC)-muag-A70-C30 construct encoding luciferase). Precomplexation of the mRNA was carried out

with PEI (800 kDa) in an N/P ratio of RNA:PEI of 0.04, 0.17, 0.67, or 1.08. Complexation of the precomplexed mRNA was carried out with the cationic polypeptide R_9H_3 (R_9H_3 = Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-His-His-His, SEQ ID NO: 6) in a molar ratio of 1:5000 (corresponding to an N/P ratio of 25). The results can be seen in Figure 6.

As a result, precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.67 and further complexed with the cationic peptide R_9H_3 (Figure 6, N/P PEI (RNA:peptide 1:5000) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone (Figure 6, N/P PEI w/o peptide) has no influence on luciferase expression (see Figure 6). The control was carried out with RNA alone (luc-RNActive 4 µg RNA) or medium without RNA (medium w/o RNA).

4.5 Transfection of stabilized luciferase mRNA constructs in CHO-cells with high molecular PEI (800 kDa) and R_9H_3 as a cationic polymer

Preparing the precomplexed complexed mRNA constructs and the transfection into HeLa-cells was generally carried out as described above under sections 3. and 4.2. Particularly, CHO-cells were transfected with a precomplexed complexed mRNA according to SEQ ID NO: 3 (mRNA concentration: 4 µg CAP-Ppluc(GC)-muag-A70-C30 construct encoding luciferase). Precomplexation of the mRNA was carried out with PEI (800 kDa) in an N/P ratio of RNA:PEI of 0.04, 0.17, or 0.67. Complexation of the precomplexed mRNA was carried out with the cationic polypeptide R_9H_3 (R_9H_3 = Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-His-His-His, SEQ ID NO: 6) in a molar ratio of 1:5000 (corresponding to an N/P ratio of 25). The results can be seen in Figure 7.

As a result, precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.67 and further complexed with the cationic peptide R_9H_3 (Figure 7, N/P PEI (RNA:peptide 1:5000) leads to a significantly increased luciferase expression in CHO-cells. The control was carried out with RNA alone (luc-RNActive 4 µg RNA), peptide alone without RNA (peptide w/o RNA) and medium without RNA (medium w/o RNA).

4.6 Transfection of stabilized luciferase mRNA constructs in L929-cells with high molecular PEI (800 kDa) and R_9H_3 as a cationic polymer

Preparing the precomplexed complexed mRNA constructs and the transfection into L929-cells was generally carried out as described above under sections 3. and 4.3. Particularly, L929-cells were transfected with a precomplexed complexed mRNA according to SEQ ID NO: 3 (mRNA concentration: 4 µg CAP-Ppluc(GC)-muag-A70-C30 construct encoding luciferase). Precomplexation of the mRNA was carried out with PEI (800 kDa) in an N/P ratio of RNA:PEI of 0.04, 0.17, 0.67, 1.08 or 2.17. Complexation of the precomplexed mRNA was carried out with the cationic polypeptide R_9H_3 (R_9H_3 = Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-His-His-His, SEQ ID NO: 6) in a molar ratio of 1:5000 (corresponding to an N/P ratio of 25). The results can be seen in Figure 8.

As a result, precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.17 to 0.67 and further complexed with the cationic peptide R_9H_3 (Figure 8, N/P PEI (RNA:peptide 1:5000) leads to a significantly increased luciferase expression in L929-cells, whereas a complexation with PEI alone (Figure 8, N/P PEI w/o peptide) has no influence on luciferase expression (see Figure 8). The control was carried out with RNA alone (luc-RNActive 4 µg RNA).

4.7 Transfection of stabilized luciferase mRNA constructs in HeLa cells with high molecular PEI (800 kDa) and protamine as a cationic polymer

Preparing the precomplexed complexed mRNA constructs and the transfection into HeLa-cells was generally carried out as described above under sections 3. and 4.1. Particularly, HeLa-cells were transfected with a precomplexed complexed mRNA according to SEQ ID NO: 3 (mRNA concentration: 4 µg CAP-Ppluc(GC)-muag-A70-C30 construct encoding luciferase). Precomplexation of the mRNA was carried out with PEI (800 kDa) in an N/P ratio of RNA:PEI of 0.04, 0.17, 0.67, or 1.08. Complexation of the precomplexed mRNA was carried out with the cationic polypeptide protamine in a mass ratio of 8:1 (corresponding to a molar ratio of 1:35 and an N/P ratio of 0.12). The results can be seen in Figure 9.

As a result, precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.17 and further complexed with the cationic peptide protamine in a mass ratio of 8:1 (corresponding to a molar ratio of 1:35 and an N/P ratio of 0.12) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone (Figure 9, w/o peptide) has no influence on luciferase expression.

4.8 Transfection of stabilized luciferase mRNA constructs in HeLa cells with either high molecular PEI (800 kDa) or low molecular PEI (25 kDa) and R_9H_3 as a cationic polymer

Preparing the precomplexed complexed mRNA constructs and the transfection into HeLa-cells was generally carried out as described above under sections 3. and 4.1. Particularly, HeLa-cells were transfected with a precomplexed complexed mRNA according to SEQ ID NO: 3 (mRNA concentration: 4 µg CAP-Ppluc(GC)-muag-A70-C30 construct encoding luciferase). Precomplexation of the mRNA was carried out with either high-molecular PEI (800 kDa) or low-molecular PEI (25 kDa) in an N/P ratio of RNA:PEI of 0.04, 0.17, 0.67, or 1.08. Complexation of the precomplexed mRNA was carried out with the cationic polypeptide R_9H_3 (R_9H_3 = Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-His-His-His, SEQ ID NO: 6) in a molar ratio of 1:5000 (corresponding to an N/P ratio of 25). The results can be seen in Figure 10.

As a result, precomplexation of the luciferase encoding mRNA, which has been precomplexed either high-molecular PEI (800 kDa) in an N/P ratio of 0.04 to 0.67 or low-molecular PEI (25 kDa) in an N/P ratio of 0.04, respectively, and further complexed with the cationic peptide R_9H_3 (Figure 10, N/P PEI 800 kDa (RNA:peptide 1:5000) or N/P PEI 25 kDa (RNA:peptide 1:5000)) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone (Figure 10, N/P PEI w/o) has no influence on luciferase expression (see Figure 10). The control was carried out with RNA alone (4 µg RNA), with medium without RNA medium (w/o RNA) or with peptide without RNA (peptide w/o RNA).

4.9 Transfection of stabilized luciferase mRNA constructs in HeLa cells with high molecular PEI (800 kDa) and R_9 as a cationic polymer

Preparing the precomplexed complexed mRNA constructs and the transfection into HeLa-cells was generally carried out as described above under sections 3. and 4.1. Particularly, HeLa-cells were transfected with a precomplexed complexed mRNA according to SEQ ID NO: 3 (mRNA concentration: 4 µg CAP-Ppluc(GC)-muag-A70-C30 construct encoding luciferase). Precomplexation of the mRNA was carried out with PEI (800 kDa) in an N/P ratio of RNA:PEI of 0.04, 0.17, 0.67, 1.08 or 2.17. Complexation of the precomplexed mRNA was carried out with the cationic polypeptide R_9H_3 (R_9 = Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg, SEQ ID NO: 7) in a molar ratio of 1:5000 (corresponding to an N/P ratio of 25). The results can be seen in Figure 11.

As a result, precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.67 and further complexed with the cationic peptide R_9 (Figure 11, N/P PEI (RNA:peptide 1:5000) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone (Figure 11, N/P PEI w/o) has no influence on luciferase expression (see Figure 11). The control was carried out with RNA alone (luc-RNActive 4 µg RNA) or with medium without RNA (medium w/o RNA).

4.10 Transfection of stabilized luciferase mRNA constructs in HeLa cells with high molecular PEI (800 kDa) and Lipofectamine as a cationic polymer

Preparing the precomplexed complexed mRNA constructs and the transfection into HeLa-cells was generally carried out as described above under sections 3. and 4.1. Particularly, HeLa-cells were transfected with a precomplexed complexed mRNA according to SEQ ID NO: 3 (mRNA concentration: 4 µg CAP-Ppluc(GC)-muag-A70-C30 construct encoding luciferase). Precomplexation of the mRNA was carried out with PEI (800 kDa) in an N/P ratio of RNA:PEI of 0.04, 0.17, 0.67 or 1.08. Complexation of the precomplexed mRNA was carried out with the cationic polymer Lipofectamine (not Lipofectamine 2000) in an RNA:Lipofectamine ratio of 1µg:1µl. Lipofectamine™ Reagent is suitable for the transfection of DNA into eukaryotic cells (1), and is a 3:1 (w/w) liposome formulation of the polycationic lipid

2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,Ndimethyl- 1-propanaminium trifluoroacetate (DOSPA) and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) in membrane-filtered water. The results of the experiment can be seen in Figure 12.

As a result, precomplexation of the luciferase encoding mRNA, which has been precomplexed with PEI (800 kDa) in an N/P ratio of 0.04 to 0.17 and further complexed with the cationic polymer Lipofectamine (not Lipofectamine 2000) in an RNA:Lipofectamine ratio of 1µg:1µl (see Figure 12) leads to a significantly increased luciferase expression in HeLa-cells, whereas a complexation with PEI alone has no influence on luciferase expression (see Figure 12). The control was carried out with RNA alone (luc-RNActive 4 µg RNA) or with medium without RNA (w/o RNA).

In this context it is to be emphasized, that according to Hattori et al (2007, supra) addition of PEI in an N/P ratio of at least 1 was assumed to enhance transfection efficiency of the cationic lipid lipofectamine. However, we were able to show that addition of PEI in an N/P ratio of about 1 leads to an even worse transfection rate of the cationic lipid lipofectamine.

Claims

1. At least one "complexed precomplexed nucleic acid", wherein a nucleic acid (molecule) has been precomplexed in a first step with PEI in an N/P ratio between 0.05 and 2, and the precomplexed nucleic acid (molecule) has been further complexed in a second step with a cationic or polycationic compound.

2. The at least one "complexed precomplexed nucleic acid" according to claim 1, wherein the nucleic acid (molecule) is an mRNA, a siRNA, an antisense RNA or an immunostimulatory RNA.

3. The at least one "complexed precomplexed nucleic acid" according to claim 1 or 2, wherein the nucleic acid (molecule) has been precomplexed in a first step with PEI in an N/P ratio of about 0.05 to 1, even more preferably in an N/P ratio of about 0.1 to 1.

4. The at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 3, wherein the nucleic acid (molecule) has been precomplexed in a first step with a high-molecular PEI, selected from a high-molecular linear polyethyleneimine (LPEI) or a high-molecular branched polyethleneimine (BPEI), having an average molecular weight of about 1 to about 1.600 kDa, more preferably selected from PEI having an average molecular weight of about 5 to about 1,500 kDa, even more preferably selected from a high-molecular LPEI or BPEI having an average molecular weight of about 10 to about 1.000 kDa, most preferably a high-molecular LPEI or BPEI having an average molecular weight of about 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or about 1.000 kDa, or selected from a high-molecular LPEI or BPEI of a molecular range formed by two of any of the values as mentioned above.

5. The at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 4, wherein the precomplexed nucleic acid molecule is complexed with the cationic or polycationic compound in an N/P ratio of about 0.1 to 50, preferably in a range of about 0.5 to 50, even more preferably in the range of about 10-50 and

most preferably in the range of about 25 to 50 of RNA:cationic or polycationic compound.

6. The at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 5, wherein the cationic or polycationic compound is selected from a cationic or polycationic compound, suitable for complexing and thereby stabilizing the at least one precomplexed nucleic acid, including cationic or polycationic peptides or proteins, including protamine, nucleoline, spermin or spermidine, poly-L-lysine (PLL), poly-arginine, basic polypeptides, cell penetrating peptides (CPPs), including HIV-binding peptides, Tat, HIV-1 Tat (HIV), Tat-derived peptides, penetratin, VP22 derived or analog peptides, HSV VP22 (Herpes simplex), MAP, KALA or protein transduction domains, including PTDs, PpT620, prolin-rich peptides, arginine-rich peptides, lysine-rich peptides, MPG-peptide(s), Pep-1, L-oligomers, calcitonin peptide(s), Antennapedia-derived peptides (*Drosophila antennapedia*), including pAntp, pIsl, FGF, lactoferrin, transportan, buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, or histones, cationic or polycationic proteins or peptides selected from proteins or peptides having the following total formula: $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$, wherein l + m + n +o + x = 8-15, and l, m, n or o independently of each other may be any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, provided that the overall content of Arg, Lys, His and Orn represents at least 50% of all amino acids of the oligopeptide; and Xaa may be any amino acid selected from native (= naturally occurring) or non-native amino acids except of Arg, Lys, His or Orn; and x may be any number selected from 0, 1, 2, 3 or 4, provided, that the overall content of Xaa does not exceed 50 % of all amino acids of the oligopeptide, including oligoarginines Arg_7, Arg_8, Arg_9, Arg_7, H_3R_9, R_9H_3, $H_3R_9H_3$, YSSR$_9$SSY, $(RKH)_4$, Y$(RKH)_2$R, or selected from cationic polysaccharides, including chitosan, polybrene, cationic polymers, cationic lipids, including DOTMA: [1-(2,3-sioleyloxy)propyl)]-N,N,N-trimethylammonium chloride, DMRIE, di-C14-amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, dioleyl phosphatidylethanol-amine (DOPE), DOSPA, DODAB, DOIC, DMEPC, dioctadecylamidoglicylspermin (DOGS), dimyristo-oxypropyl dimethyl hydroxyethyl ammonium bromide (DIMRI), dioleoyloxy-3-(trimethylammonio)propane (DOTAP), DC-6-14: O,O-ditetradecanoyl-N-(α-trimethylammonioacetyl)diethanolamine

chloride, rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride (CLIP1), rac-[2(2,3-dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium (CLIP6), rac-[2(2,3-dihexadecyloxypropyl-oxysuccinyloxy)ethyl]-trimethylammonium (CLIP9), oligofectamine, or selected from cationic or polycationic polymers, including modified polyaminoacids, including β-aminoacid-polymers or reversed polyamides, modified polyethylenes, including PVP (poly(N-ethyl-4-vinylpyridinium bromide)), modified acrylates, including pDMAEMA (poly(dimethylaminoethyl methylacrylate)), modified amidoamines, including pAMAM (poly(amidoamine)), modified polybetaaminoester (PBAE), including diamine end modified 1,4 butanediol diacrylate-co-5-amino-1-pentanol polymers, dendrimers, including polypropylamine dendrimers or pAMAM based dendrimers, poly(propyleneimine), polyallylamine, sugar backbone based polymers, including cyclodextrin based polymers, dextran based polymers, Chitosan, silan backbone based polymers, including PMOXA-PDMS copolymers, blockpolymers consisting of a combination of one or more cationic blocks of a cationic polymer as defined above, and of one or more hydrophilic- or hydrophobic blocks, including polyethyleneglycol.

7. The at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 6, wherein the nucleic acid (molecule) is a coding nucleic acid molecule, preferably an mRNA, encoding at least one therapeutically active protein or peptide, including apoptotic factors or apoptosis related proteins, adjuvant protein, including human adjuvant proteins or from pathogenic adjuvant proteins, in particular from bacterial adjuvant proteins, protozoa proteins, viral proteins, fungal proteins, or pathogenic adjuvant proteins, at least one antigen, including tumor antigens, pathogenic antigens or pathogens, animal antigens, viral antigens, protozoal antigens, bacterial antigens, autoimmune (self-)antigens, at least one allergen or allergen antigen, including allergy antigens from animals, plants, fungi, bacteria, grasses, pollens, molds, drugs, or environmental triggers, and/or at least one antibody.

8. The at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 7, wherein the nucleic acid (molecule) is a GC-stabilized mRNA.

9. The at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 8, wherein the nucleic acid (molecule) is an mRNA and the G/C content of the coding region of the GC-stabilized mRNA is increased compared with the G/C content of the coding region of the native mRNA, the coded amino acid sequence of the GC-stabilized mRNA not being altered compared with the coded amino acid sequence of the native mRNA.

10. The at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 9, wherein the nucleic acid (molecule) has a 5' cap structure and/or a poly(A) tail, preferably of 10 to 200 adenosine nucleotides, and/or a poly(C) tail, preferably of 10 to 200 cytosine nucleotides, and/or at least one IRES and/or at least one 5' and/or 3' stabilizing sequence.

11. Pharmaceutical composition, comprising the at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 10, and optionally a pharmaceutically acceptable carrier, adjuvant, and/or vehicle.

12. Pharmaceutical composition according to claim 11, wherein the pharmaceutical composition is a vaccine.

13. A method for transfection of a cell or an organism, comprising the following steps:
    a. optionally preparing and/or providing the at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 10, wherein a nucleic acid (molecule) has been precomplexed in a first step with PEI in an N/P ratio between 0.05 and 2, and the precomplexed nucleic acid (molecule) has been further complexed in a second step with a cationic or polycationic compound;
    b. transfecting a cell, a tissue or an organism using the at least one "complexed precomplexed nucleic acid" prepared and/or provided according to step a).

14. Method according to claim 13, wherein the organism is a mammal, selected from the group comprising humans, goat, cattle, swine, dog, cat, donkey, monkey, ape or rodents, including mouse, hamster and rabbit.

15. Use of the at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 10 for transfecting a cell or an organism.

16. Use the at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 10 for preparing a pharmaceutical composition or a vaccine for treating or ameliorating a disease selected from cancer or tumor diseases, infectious diseases, preferably (viral, bacterial or protozoological) infectious diseases, autoimmune diseases, allergies or allergic diseases, monogenetic diseases, i.e. (hereditary) diseases, or genetic diseases in general, cardiovascular diseases and/or neural diseases.

17. Kit (of parts) comprising the at least one "complexed precomplexed nucleic acid" according to any of claims 1 to 10, and/or the pharmaceutical composition or vaccine according to any of claims 11 to 12, and optionally technical instructions with information on the administration and dosage of the immunostimulatory composition and/or the pharmaceutical composition.

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GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
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CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
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GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
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AUU

Figure 1

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```

Figure 2

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Figure 3

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Figure 4

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UGC GUG CGG GGG CCG AUG AUC AUG AGC GGC UAC GUG AAC AAC CCG
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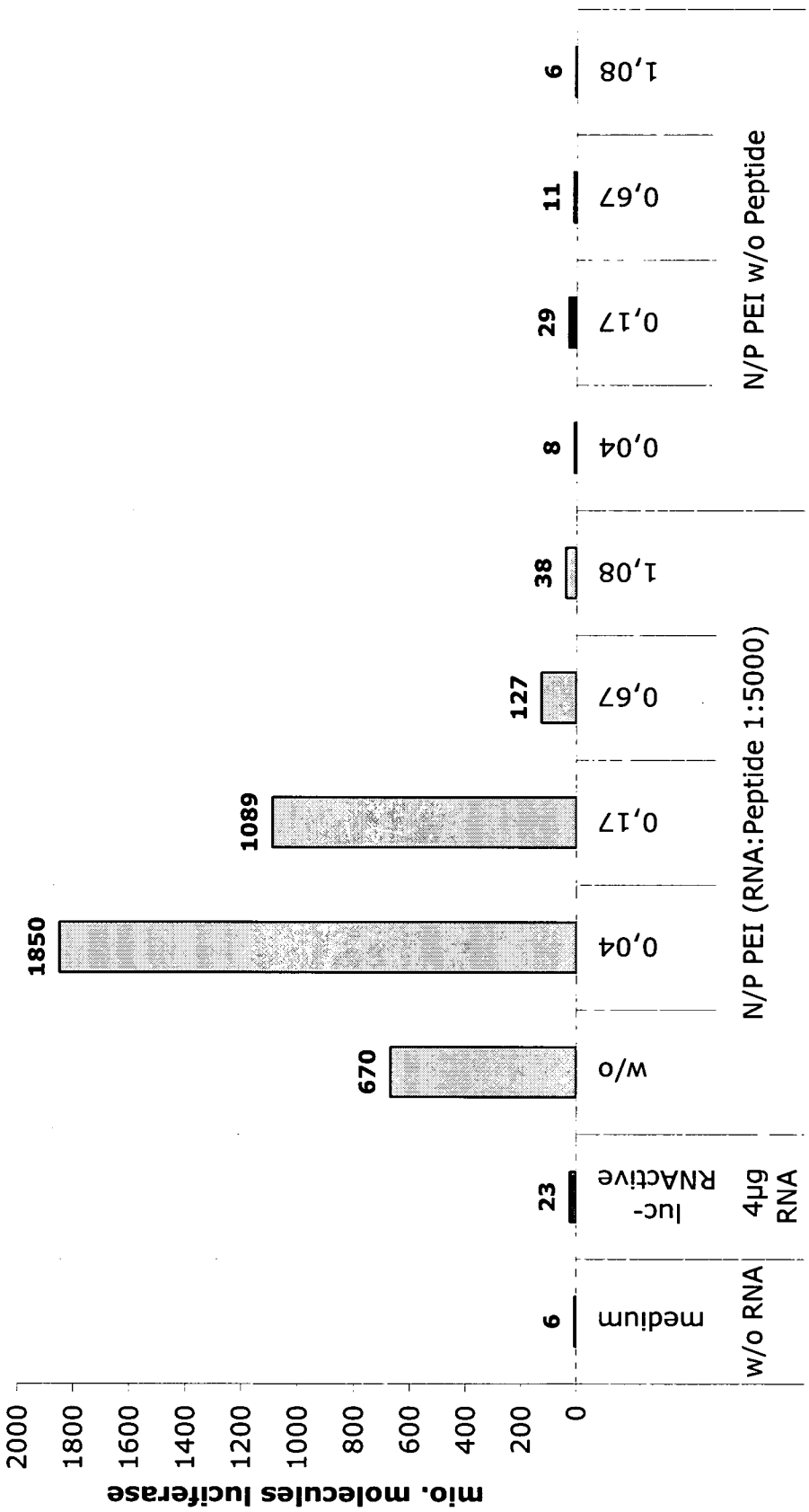
AAG GCC AAG AAG GGC GGC AAG AUC GCC GUG UAA

Figure 5


Effect of PEI addition to R9H3 on the expression of luciferase in HeLa cells
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1800
1600
1400
1200
1000
800
600
400
200
0
w/o RNA
4µg RNA
medium
luc-RNActive
w/o
N/P PEI (RNA:Peptide 1:5000)
N/P PEI w/o Peptide
6
23
670
1850
1089
127
38
8
29
11
6
0,04
0,17
0,67
1,08
0,04
0,17
0,67
1,08

Figure 6

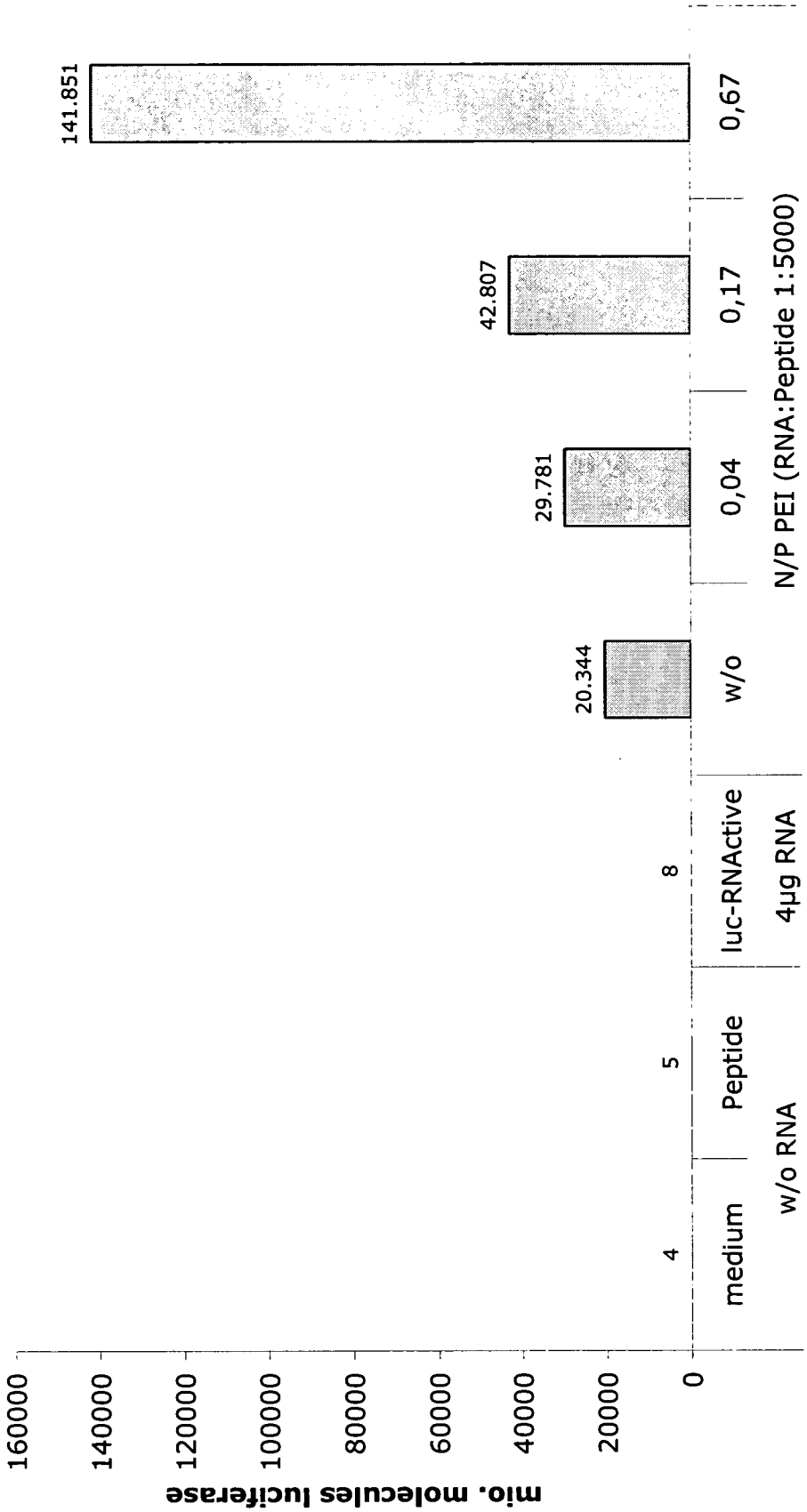
Effect of PEI addition to R9H3 to the expression of luciferase in CHO cells
mio. molecules luciferase
160000
140000
120000
100000
80000
60000
40000
20000
0
4
5
8
medium
Peptide
luc-RNActive
w/o RNA
4µg RNA
20.344
29.781
42.807
141.851
w/o
0,04
0,17
0,67
N/P PEI (RNA:Peptide 1:5000)

Figure 7

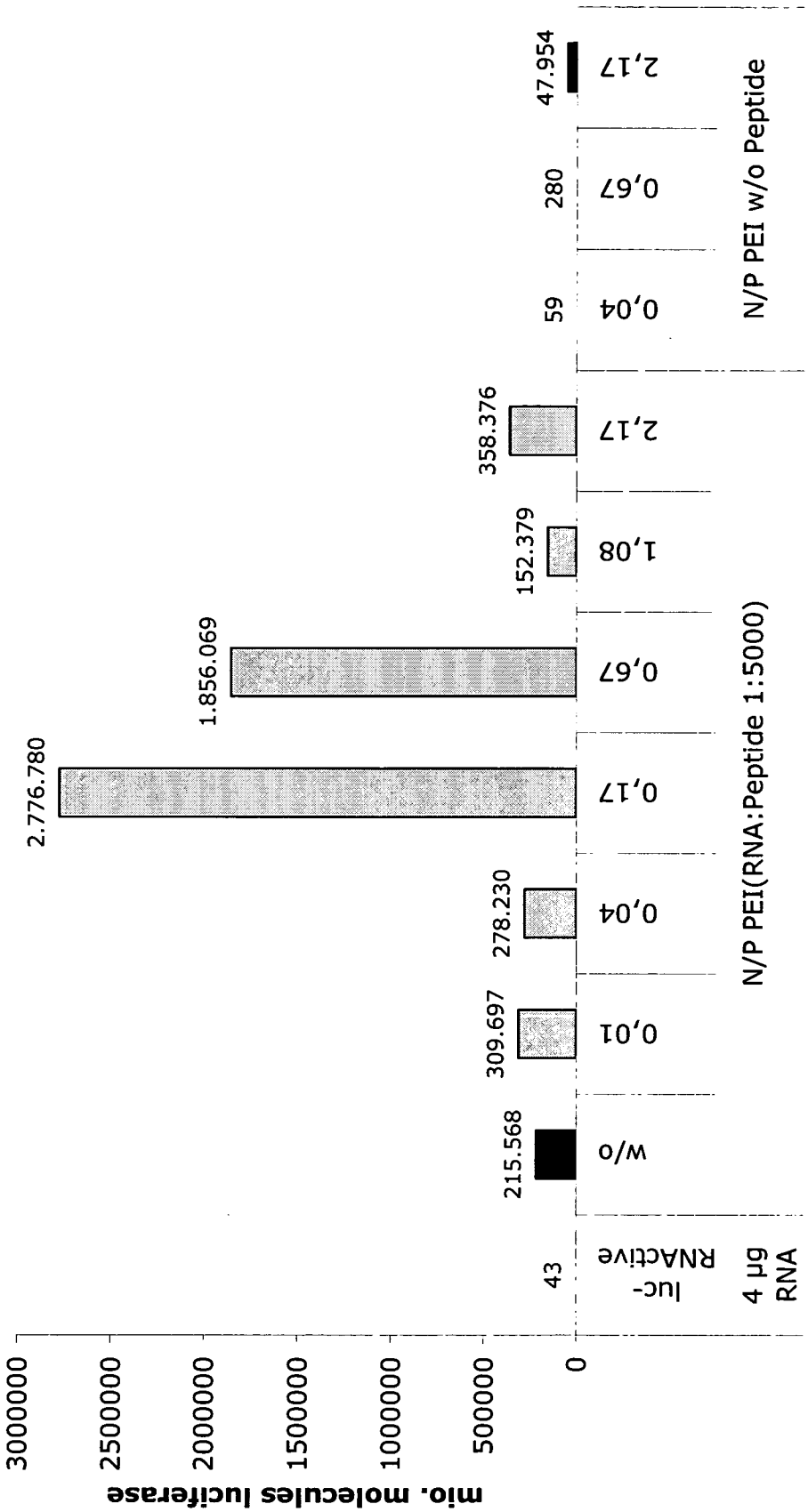
Effect of PEI addition to R9H3 on the expression of luciferase in L929 cells
mio. molecules luciferase
3000000
2500000
2000000
1500000
1000000
500000
0
4 µg RNA
luc-RNActive
43
w/o
215.568
0,01
309.697
0,04
278.230
0,17
2.776.780
0,67
1.856.069
1,08
152.379
2,17
358.376
N/P PEI(RNA:Peptide 1:5000)
0,04
59
0,67
280
2,17
47.954
N/P PEI w/o Peptide

Figure 8

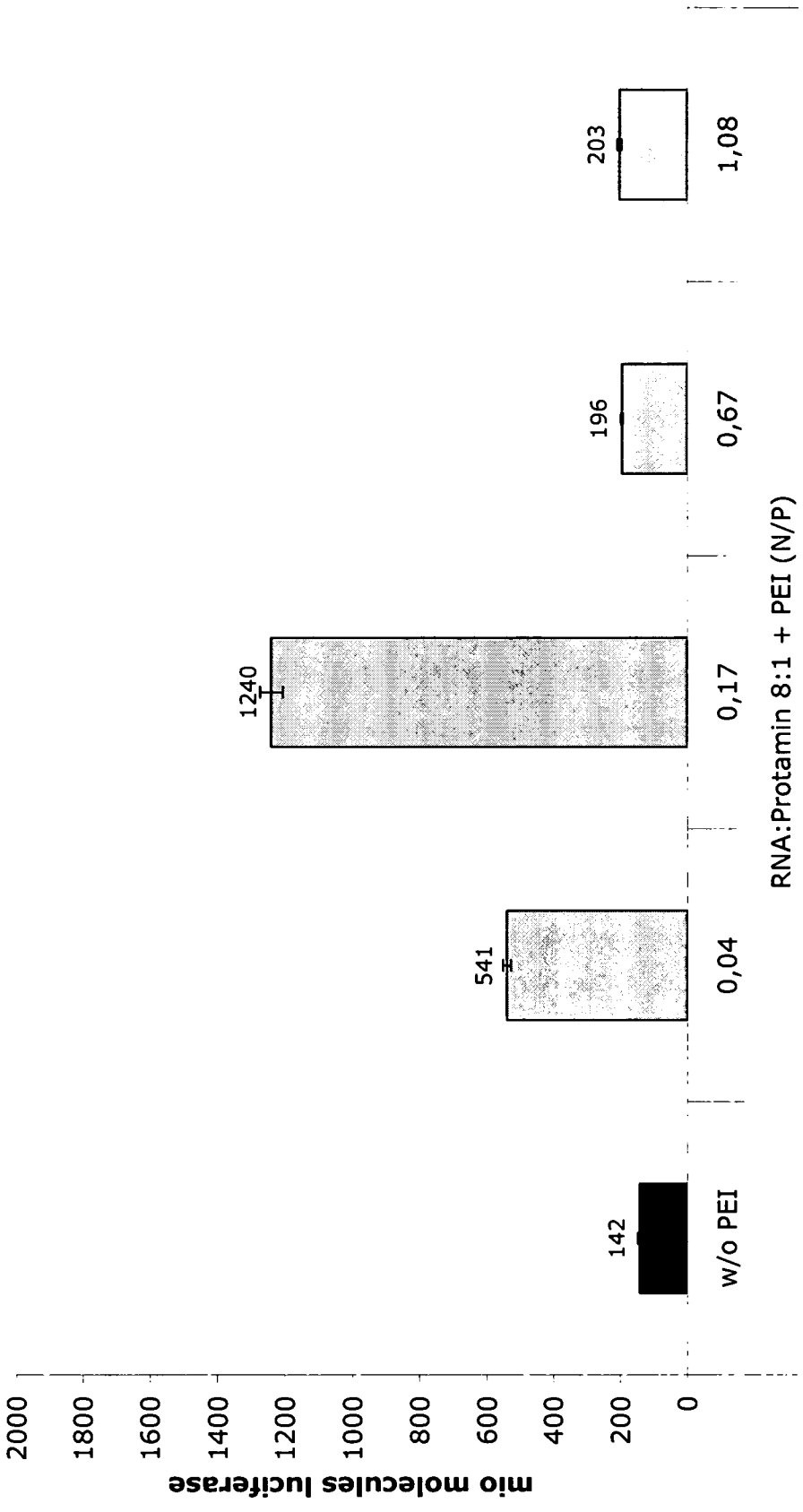
Effect of PEI addition to protamine on the expression of luciferase in HeLa cells
mio molecules luciferase
2000
1800
1600
1400
1200
1000
800
600
400
200
0
142
541
1240
196
203
w/o PEI
0,04
0,17
0,67
1,08
RNA:Protamin 8:1 + PEI (N/P)

Figure 9

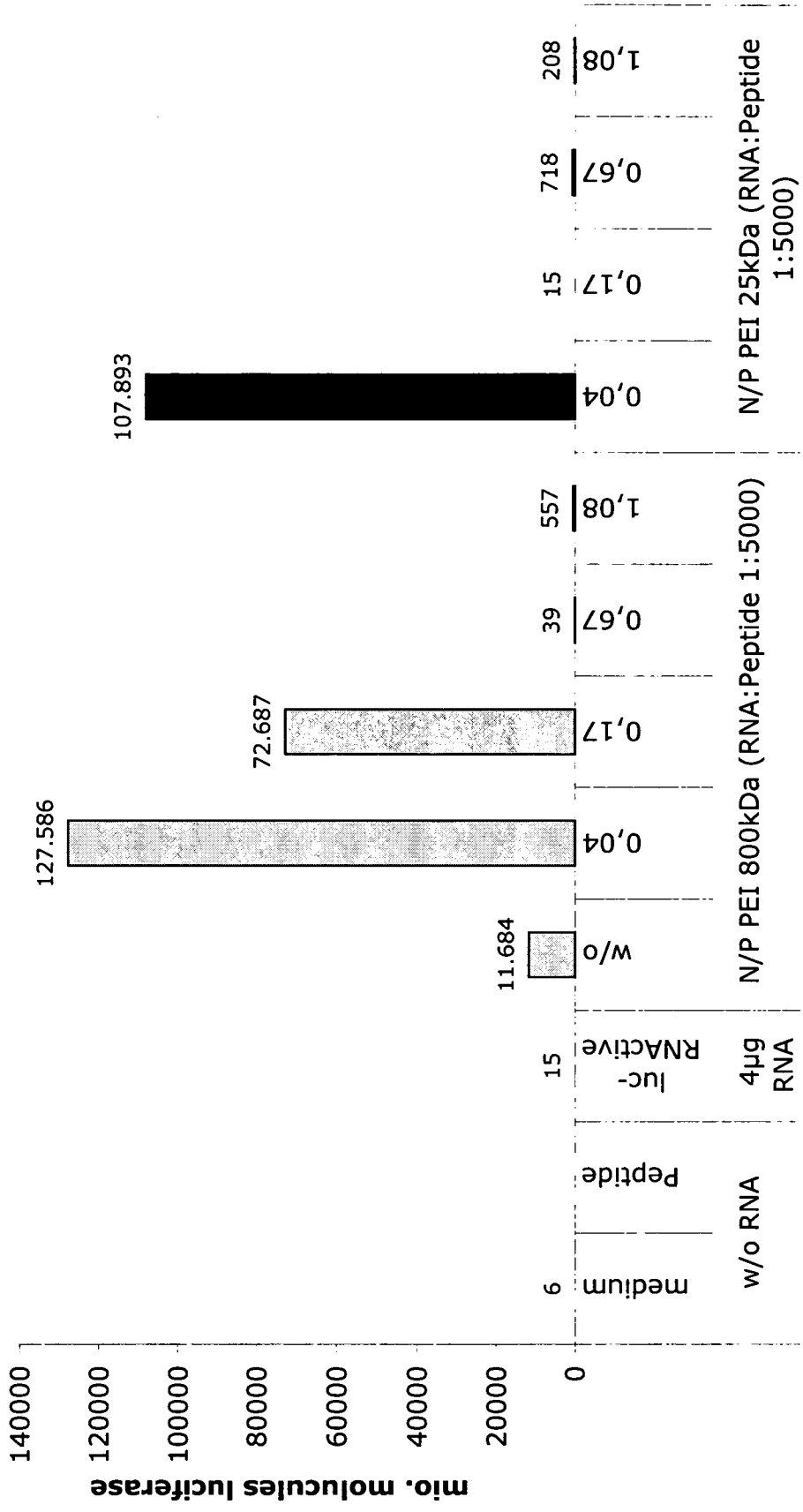
Effect of PEI addition to R9H3 on the expression of luciferase in HeLa cells
mio. molucules luciferase
140000
120000
100000
80000
60000
40000
20000
0
medium
Peptide
Luc-RNActive
w/o
0,04
0,17
0,67
1,08
0,04
0,17
0,67
1,08
w/o RNA
4µg RNA
N/P PEI 800kDa (RNA:Peptide 1:5000)
N/P PEI 25kDa (RNA:Peptide 1:5000)
6
15
11.684
127.586
72.687
39
557
107.893
15
718
208

Figure 10

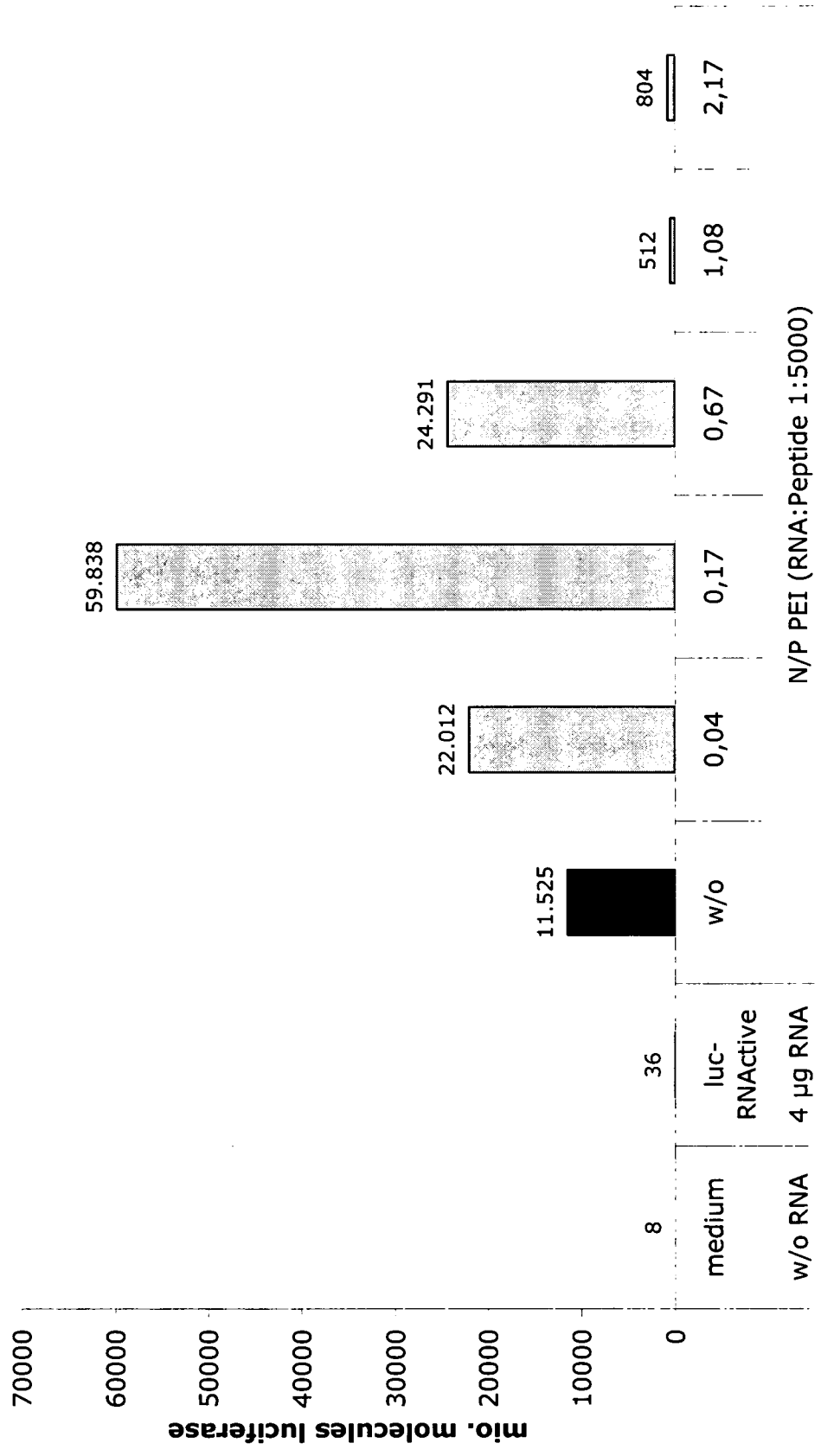
Effect of PEI addition to R9 on the expression of luciferase in HeLa cells
mio. molecules luciferase
70000
60000
50000
40000
30000
20000
10000
0
11.525
22.012
59.838
24.291
804
512
8
36
0,04
0,17
0,67
1,08
2,17
w/o RNA
medium
4 µg RNA
luc-RNActive
w/o
N/P PEI (RNA:Peptide 1:5000)

Figure 11

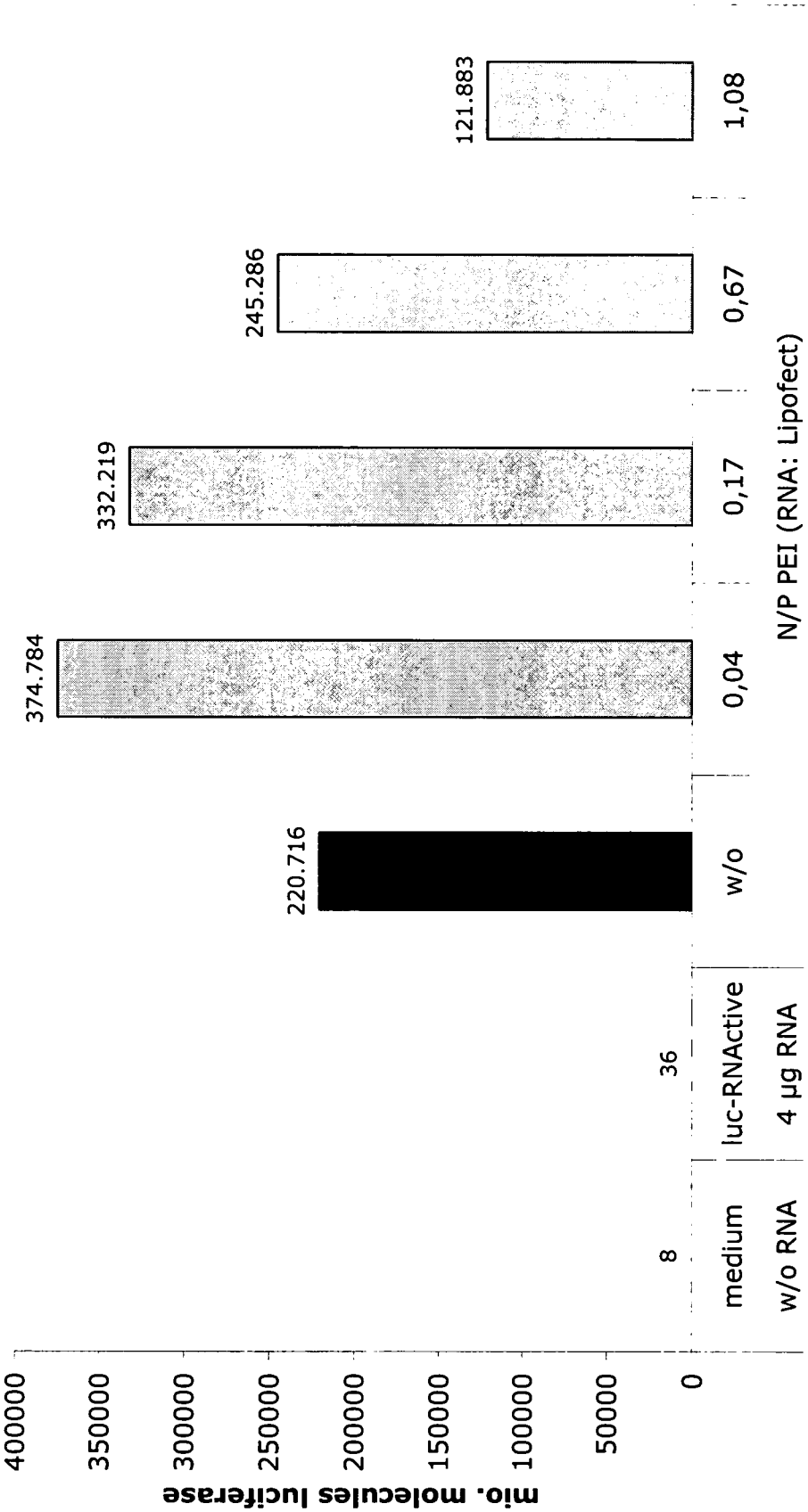
Effect of PEI addition to Lipofectamine on the expression of luciferase in HeLa cells
mio. molecules luciferase
400000
350000
300000
250000
200000
150000
100000
50000
0
8
36
medium
w/o RNA
luc-RNActive
4 µg RNA
220.716
374.784
332.219
245.286
121.883
w/o
0,04
0,17
0,67
1,08
N/P PEI (RNA: Lipofect)

Figure 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/000886

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/87 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HATTORI YOSHIYUKI ET AL: "Low-molecular-weight polyethylenimine enhanced gene transfer by cationic cholesterol-based nanoparticle vector" BIOLOGICAL & PHARMACEUTICAL BULLETIN, vol. 30, no. 9, September 2007 (2007-09), pages 1773-1778, XP002551956 ISSN: 0918-6158 page 1773 abstract page 1774, left-hand column, paragraph 2 page 1775, left-hand column; figure 2 page 1777; figure 6 ----- -/--	1-17

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 October 2009	03/11/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sitch, David

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/000886

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/024708 A (UNIV PENNSYLVANIA [US]; KARIKO KATALIN [US]; WEISSMAN DREW [US]) 1 March 2007 (2007-03-01) page 22, line 17 - line 26 page 55 - page 67; examples 10-25 claims 1-3	
A	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; December 1999 (1999-12), DHEUR S ET AL: "Polyethylenimine but not cationic lipid improves antisense activity of 3'-capped phosphodiester oligonucleotides." XP002551957 Database accession no. NLM10645777 abstract & ANTISENSE & NUCLEIC ACID DRUG DEVELOPMENT DEC 1999, vol. 9, no. 6, December 1999 (1999-12), pages 515-525, ISSN: 1087-2906	
A	DATABASE EMBASE [Online] ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL; 2002, THORPE P ET AL: "Optimising gene repair strategies in cell culture" XP002551958 Database accession no. EMB-2002196988 abstract & GENE THERAPY 2002 GB, vol. 9, no. 11, 2002, pages 700-702, ISSN: 0969-7128	
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 2004 (2004-07), BRUS CAROLA ET AL: "Physicochemical and biological characterization of polyethylenimine-graft-poly(ethylene glycol) block copolymers as a delivery system for oligonucleotides and ribozymes" XP002551959 Database accession no. PREV200400398678 abstract & BIOCONJUGATE CHEMISTRY, vol. 15, no. 4, July 2004 (2004-07), pages 677-684, ISSN: 1043-1802	

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/000886

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AIGNER A ET AL: "DELIVERY OF UNMODIFIED BIOACTIVE RIBOZYMES BY AN RNA-STABILIZING POLYETHYLENIMINE (LMW-PEL) EFFICIENTLY DOWN-REGULATES GENE EXPRESSION" GENE THERAPY, MACMILLAN PRESS LTD., BASINGSTOKE, GB, vol. 9, no. 24, 1 December 2002 (2002-12-01), pages 1700-1707, XP009029430 ISSN: 0969-7128 page 1700 abstract	
A	TOENGES LARS ET AL: "Stearylated octaarginine and artificial virus-like particles for transfection of siRNA into primary rat neurons" RNA, COLD SPRING HARBOR LABORATORY PRESS, WOODBURY, NY, US, vol. 12, no. 7, 1 July 2006 (2006-07-01), pages 1431-1438, XP002481136 ISSN: 1355-8382 page 1431 abstract	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2009/000886

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007024708 A	01-03-2007	EP 1979364 A2	15-10-2008