RNA containing composition for treatment of tumor diseases

Introduction

The present invention relates to RNA containing compositions for use in the treatment or prophylaxis of tumor and/or cancer diseases, to a pharmaceutical composition, to a kit and to uses of the RNA containing compositions for the treatment or prophylaxis of tumor and/or cancer diseases.

Cancer, also known as malignant tumor, describes a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. In 2012, about 14.1 million new cases of cancer occurred globally (not including skin cancer other than melanoma).

The standard treatments of cancer include chemotherapy, radiation und surgery, wherein these treatments are applied individually or in combination. Other treatments apply cancer immunotherapy which is focused on stimulating the immune system through vaccination or adoptive cellular immunotherapy to elicit an anti-tumor response.

Some approaches use gene therapy and genetic vaccination for treatment of cancer or other tumor diseases. Gene therapy and genetic vaccination are molecular medicine methods which are based on the introduction of nucleic acids into cells or into tissues of a patient. Subsequently the information coded by the nucleic acids introduced is processed in the organism, i.e. resulting in expression of a therapeutic peptide or protein or expression of an antigen which is coded by the nucleic acids.

Conventional gene therapeutic methods, including gene therapy and genetic vaccination are based on the use of DNA molecules in order to transfer the desired genetic information into the cell.

Various methods have been developed for introducing DNA into cells, such as calcium phosphate transfection, polybrene transfection, protoplast fusion, electroporation, microinjection and lipofection. DNA viruses may likewise be used as a DNA vehicle achieving a very high transfection rate. The use of DNA entails the risk of the DNA being inserted into an intact gene of the host cell's genome by e.g. recombination. In this case the affected gene may be mutated and inactivated or may give rise to misinformation. Another risk of using DNA as a pharmaceutical agent is the risk of inducing pathogenic anti-drug antibodies(anti-DNA antibodies) in the patient, which may result in a (possibly fatal) immune response.

The use of RNA as a gene therapeutic agent or genetic vaccine is substantially safer, because RNA does not involve the risk of being integrated into the genome inducing an undesired pathogenic induction of anti-drug antibodies.

Thus RNA expression systems have considerable advantages over DNA expression systems in gene therapy and in genetic vaccination although it is known in the prior art or rather assumed for a long time that the instability of mRNA or of RNA in general may be problem in the application of medical methods based on RNA expression systems.

The instability of RNA is in particular due to RNA-degrading enzymes (ribonucleases - RNases). There are also many further processes which destabilize RNA, wherein interaction between the RNA and proteins often appears to play a crucial role. Some measures for increasing the stability of RNA have been proposed, so enabling the use thereof as a gene therapy agent or RNA vaccine.

For solving the problem of *ex vivo* RNA stability the European patent application EP 1 083 232 A1 describes a method for introducing RNA, in particular mRNA, into cells and organisms, in which the RNA forms a complex with a cationic peptide or protein.

The application of mRNA is known for the treatment and/or prophylaxis of cancer. For example the international patent application WO 03/051401 A2 describes a pharmaceutical composition comprising at least one mRNA, which contains at least one region that codes for an antigen from a tumor, combined with an aqueous solvent and preferably with a cytokine e.g. GM-CSF. The pharmaceutical composition is proposed to be used for therapy and/or prophylaxis against cancer.

The international patent application WO 2006/008154 A1 discloses an mRNA mixture for vaccinating against tumor diseases, wherein at least one type of mRNA contains at least one tumor antigen-coding region. At least one other mRNA contains at least one type of an immunogenic protein-coding region.

Nevertheless there is still a need for an effective treatment of tumor diseases and especially for the treatment of cancer. Therefore it is the object of the underlying invention to provide an approach for effective treatment of tumor diseases wherein tumor tissue and cancer cells are specifically destroyed.

This object is solved by the subject matter of the claims. Particularly, the object underlying the present invention is solved according to a first aspect by an RNA containing composition for use in the treatment or prophylaxis of tumor and/or cancer diseases. According to further aspects of the invention the object is solved by a pharmaceutical composition, by a kit or kit of parts, and by a method of treatment of tumor or cancer diseases.

Definitions:

For the sake of clarity and readability the following scientific background information and definitions are provided. Any technical features disclosed thereby can be part of each and every embodiment of the invention. Additional definitions and explanations can be provided in the context of this disclosure.

Immune system: The immune system may protect organisms from infection. If a pathogen breaks through a physical barrier of an organism and enters this organism, the innate immune system provides an immediate, but non-specific response. If pathogens evade this innate response, vertebrates possess a second layer of protection, the adaptive immune system. Here, the immune system adapts its response during an infection to improve its recognition of the pathogen. This improved response is then retained after the pathogen has been eliminated, in the form of an immunological memory, and allows the adaptive immune system to mount faster and stronger attacks each time this pathogen is encountered. According to this, the immune system comprises

the innate and the adaptive immune system. Each of these two parts contains so called humoral and cellular components.

Immune response: An immune response may typically either be a specific reaction of the adaptive immune system to a particular antigen (so called specific or adaptive immune response) or an unspecific reaction of the innate immune system (so called unspecific or innate immune response).

Adaptive immune system: The adaptive immune system is composed of highly specialized, systemic cells and processes that eliminate or prevent pathogenic growth. The adaptive immune response provides the vertebrate immune system with the ability to recognize and remember specific pathogens (to generate immunity), and to mount stronger attacks each time the pathogen is encountered. The system is highly adaptable because of somatic hypermutation (a process of increased frequency of somatic mutations), and V(D)J recombination (an irreversible genetic recombination of antigen receptor gene segments). This mechanism allows a small number of genes to generate a vast number of different antigen receptors, which are then uniquely expressed on each individual lymphocyte. Because the gene rearrangement leads to an irreversible change in the DNA of each cell, all of the progeny (offspring) of that cell will then inherit genes encoding the same receptor specificity, including the Memory B cells and Memory T cells that are the keys to long-lived specific immunity. Immune network theory is a theory of how the adaptive immune system works, that is based on interactions between the variable regions of the receptors of T cells, B cells and of molecules made by T cells and B cells that have variable regions.

Adaptive immune response: The adaptive immune response is typically understood to be antigen-specific. Antigen specificity allows for the generation of responses that are tailored to specific antigens, pathogens or pathogen-infected cells. The ability to mount these tailored responses is maintained in the body by "memory cells". Should a pathogen infect the body more than once, these specific memory cells are used to quickly eliminate it. In this context, the first step of an adaptive immune response is the activation of naïve antigen-specific T cells or different immune cells able to induce an antigen-specific immune response by antigen-presenting cells. This occurs in the lymphoid tissues and organs through which naïve T cells are constantly passing. Cell types that can serve as antigen-presenting cells are *inter alia* dendritic cells, macrophages, and B cells. Each of these cells has a distinct function in eliciting immune responses. Dendritic cells take

up antigens by phagocytosis and macropinocytosis and are stimulated by contact with e.g. a foreign antigen 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 or other appropriate stimuli to express MHC molecules. The unique ability of B cells to bind and internalize soluble protein antigens via their receptors may also 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 do not recognize and bind antigen directly, but instead recognize short peptide fragments e.g. of pathogen-derived protein antigens, which are bound to MHC molecules on the surfaces of other cells.

Cellular immunity/cellular immune response: Cellular immunity relates typically to the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. In a more general way, cellular immunity is not related to antibodies but to the activation of cells of the immune system. A cellular immune response is characterized e.g. by activating antigen-specific cytotoxic T-lymphocytes that are able to induce apoptosis in body cells displaying epitopes of an antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens; activating macrophages and natural killer cells, enabling them to destroy pathogens; and stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Humoral immunity/humoral immune response: Humoral immunity refers typically to antibody production and the accessory processes that may accompany it. A humoral immune response may be typically characterized, e.g., by Th2 activation and cytokine production, germinal center formation and isotype switching, affinity maturation and memory cell generation. Humoral immunity also typically may refer to the effector functions of antibodies, which include pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination.

Innate immune system: The innate immune system, also known as non-specific immune system, comprises the cells and mechanisms that defend the host from infection by other organisms in a non-specific manner. This means that the cells of the innate system recognize and respond to pathogens in a generic way, but unlike the adaptive immune system, it does not confer long-lasting or protective immunity to the host. The innate immune system may be e.g. activated by ligands of pathogen-associated molecular patterns (PAMP) receptors, e.g. Toll-like receptors (TLRs) or other auxiliary substances such as lipopolysaccharides, TNF-alpha, CD40 ligand, or cytokines, monokines, lymphokines, interleukins or chemokines, immunostimulatory nucleic acids, immunostimulatory RNA (isRNA), CpG-DNA, antibacterial agents, or anti-viral agents. Typically a response of the innate immune system includes recruiting immune cells to sites of infection, through the production of chemical factors, including specialized chemical mediators, called cytokines; activation of the complement cascade; identification and removal of foreign substances present in organs, tissues, the blood and lymph, by specialized white blood cells; activation of the adaptive immune system through a process known as antigen presentation; and/or acting as a physical and chemical barrier to infectious agents.

Adjuvant/adjuvant component: An adjuvant or an adjuvant component in the broadest sense is typically a (e.g. pharmacological or immunological) agent or composition that may modify, e.g. enhance, the efficacy of other agents, such as a drug or vaccine. Conventionally the term refers in the context of the invention to a compound or composition that serves as a carrier or auxiliary substance for immunogens and/or other pharmaceutically active compounds. It is to be interpreted in a broad sense and refers to a broad spectrum of substances that are able to increase the immunogenicity of antigens incorporated into or co-administered with an adjuvant in question. In the context of the present invention an adjuvant will preferably enhance the specific immunogenic effect of the active agents of the present invention. Typically, “adjuvant” or “adjuvant component” has the same meaning and can be used mutually. Adjuvants may be divided, e.g., into immuno potentiators, antigenic delivery systems or even combinations thereof.

The term “adjuvant” is typically understood not to comprise agents which confer immunity by themselves. An adjuvant assists the immune system unspecifically to enhance the antigen-specific immune response by e.g. promoting presentation of an antigen to the immune system or induction

of an unspecific innate immune response. Furthermore, an adjuvant may preferably e.g. modulate the antigen-specific immune response by e.g. shifting the dominating Th2-based antigen specific response to a more Th1-based antigen specific response or vice versa. Accordingly, an adjuvant may favourably modulate cytokine expression/secretion, antigen presentation, type of immune response etc.

Immunostimulatory/immunostimulating RNA: An immunostimulatory/immunostimulating RNA (isRNA) in the context of the invention may typically be a RNA that is able to induce an innate immune response itself. It usually does not have an open reading frame and thus does not provide a peptide-antigen or immunogen but elicits an innate immune response e.g. by binding to a specific kind of Toll-like-receptor (TLR) or other suitable receptors. _Therefore immunostimulatory/immunostimulating RNAs are preferably non-coding RNAs. However, of course also mRNAs having an open reading frame and coding for a peptide/protein (e.g. an antigenic function) may induce an innate immune response.

Antigen: The term "antigen" refers typically to a substance which may be recognized by the immune system and may be capable of triggering an antigen-specific immune response, e.g. by formation of antibodies or antigen-specific T-cells as part of an adaptive immune response. An antigen may be a protein or peptide. In this context, the first step of an adaptive immune response is the activation of naïve antigen-specific T cells by antigen-presenting cells. This occurs in the lymphoid tissues and organs through which naïve 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. By 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 molecules - MHC class I and MHC class II molecules - which differ in their structure and expression pattern on tissues of the body. CD4+ T cells bind to a MHC class II molecule and CD8+ T cells to a MHC class I molecule. MHC class I and MHC class II molecules have distinct distributions among cells that reflect the different effector functions of the T cells that recognize them. MHC class I molecules present peptides of cytosolic and nuclear origin e.g. 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 tumor antigens are presented by them. MHC class I molecules bind peptides from proteins degraded in the cytosol and transported in the endoplasmic reticulum. The CD8+ T cells that recognize MHC class I:peptide complexes at the surface of infected cells 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 naïve B cells to secrete IgM, and induce the production of weakly opsonising antibodies such as IgG1 and IgG3 (mouse) and IgG2 and IgG4 (human) as well as IgA and IgE (mouse and human).

Epitope (also called "antigen determinant"): T cell epitopes 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. B cell epitopes are typically fragments located on the outer surface of (native) protein or peptide antigens.

Vaccine: A vaccine is typically understood to be a prophylactic or therapeutic material providing at least one antigen or antigenic function. The antigen or antigenic function may stimulate the body's adaptive immune system to provide an adaptive immune response.

Antigen-providing mRNA: An antigen-providing mRNA may typically be an mRNA, having at least one open reading frame that can be translated by a cell or an organism provided with that mRNA. The product of this translation is a peptide or protein that may act as an antigen, preferably as an immunogen. The product may also be a fusion protein composed of more than one immunogen, e.g. a fusion protein that consist of two or more epitopes, peptides or proteins, wherein the epitopes, peptides or proteins may be linked by linker sequences.

Bi-/multicistronic mRNA: An bi-/multicistronic mRNA typically may have two (bicistronic) or more (multicistronic) coding sequences (cds) (also often referred to as open reading frames (ORF)). A coding sequence/an open reading frame in this context is a sequence of several nucleotide triplets (codons) that can be translated into a peptide or protein. Translation of such an mRNA yields two (bicistronic) or more (multicistronic) distinct translation products (provided the coding

sequences/ORFs are not identical). For expression in eukaryotes such mRNAs may for example comprise an internal ribosomal entry site (IRES) sequence.

5'-CAP-Structure: A 5'-CAP is typically a modified nucleotide (CAP analogue), particularly a guanine nucleotide, added to the 5' end of an mRNA molecule. Preferably, the 5'-CAP is added using a 5'-5'-triphosphate linkage (also named m7GpppN). Further examples of 5'-CAP structures include glyceryl, inverted deoxy abasic residue (moiety), 4',5' methylene nucleotide, 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide, 1,5-anhydrohexitol nucleotide, L-nucleotides, alpha-nucleotide, modified base nucleotide, threo-pentofuranosyl nucleotide, acyclic 3',4'-seco nucleotide, acyclic 3,4-dihydroxybutyl nucleotide, acyclic 3,5 dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety, 3'-3'-inverted abasic moiety, 3'-2'-inverted nucleotide moiety, 3'-2'-inverted abasic moiety, 1,4-butanediol phosphate, 3'-phosphoramidate, hexylphosphate, aminohexyl phosphate, 3'-phosphate, 3'phosphorothioate, phosphorodithioate, or bridging or non-bridging methylphosphonate moiety. These modified 5'-CAP structures may be used in the context of the present invention to modify the mRNA sequence of the inventive composition. Further modified 5'-CAP structures which may be used in the context of the present invention are CAP1 (additional methylation of the ribose of the adjacent nucleotide of m7GpppN), CAP2 (additional methylation of the ribose of the 2nd nucleotide downstream of the m7GpppN), CAP3 (additional methylation of the ribose of the 3rd nucleotide downstream of the m7GpppN), CAP4 (additional methylation of the ribose of the 4th nucleotide downstream of the m7GpppN), ARCA (anti-reverse CAP analogue), modified ARCA (e.g. phosphothioate modified ARCA), inosine, N1-methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

In the context of the present invention, a 5' cap structure may also be formed in chemical RNA synthesis or RNA *in vitro* transcription (co-transcriptional capping) using cap analogues, or a cap structure may be formed *in vitro* using capping enzymes (e.g., commercially available capping kits)

Cap analogue: A cap analogue refers to a non-polymerizable di-nucleotide that has cap functionality in that it facilitates translation or localization, and/or prevents degradation of the RNA molecule when incorporated at the 5' end of the RNA molecule. Non-polymerizable means that the

cap analogue will be incorporated only at the 5'terminus because it does not have a 5' triphosphate and therefore cannot be extended in the 3' direction by a template-dependent RNA polymerase.

Cap analogues include, but are not limited to, a chemical structure selected from the group consisting of m7GpppG, m7GpppA, m7GpppC; unmethylated cap analogues (e.g., GpppG); dimethylated cap analogue (e.g., m2,7GpppG), trimethylated cap analogue (e.g., m2,2,7GpppG), dimethylated symmetrical cap analogues (e.g., m7Gpppm7G), or anti reverse cap analogues (e.g., ARCA; m7,2'OmeGpppG, m7,2'dGpppG, m7,3'OmeGpppG, m7,3'dGpppG and their tetraphosphate derivatives) (Stepinski et al., 2001. RNA 7(10):1486-95).

Further cap analogues have been described previously (US 7,074,596, WO 2008/016473, WO 2008/157688, WO 2009/149253, WO 2011/015347, and WO 2013/059475). The synthesis of N^7-(4-chlorophenoxyethyl) substituted dinucleotide cap analogues has been described recently (Kore et al. (2013) Bioorg. Med. Chem. 21(15): 4570-4).

Fragments of proteins: "Fragments" of proteins or peptides in the context of the present invention may, typically, comprise a sequence of a protein or peptide as defined herein, which is, with regard to its amino acid sequence (or its encoded nucleic acid molecule), N-terminally and/or C-terminally truncated compared to the amino acid sequence of the original (native) protein (or its encoded nucleic acid molecule). Such truncation may thus occur either on the amino acid level or correspondingly on the nucleic acid level. A sequence identity with respect to such a fragment as defined herein may therefore preferably refer to the entire protein or peptide as defined herein or to the entire (coding) nucleic acid molecule of such a protein or peptide. In the context of antigens such fragment may have 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. 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 proteins or peptides (e.g. in the context of antigens) may comprise at least one epitope of those proteins or

peptides. Furthermore also domains of a protein, like the extracellular domain, the intracellular domain or the transmembrane domain and shortened or truncated versions of a protein may be understood to comprise a fragment of a protein. Preferably, a fragment of a protein comprises a functional fragment of the protein, which means that the fragment exerts the same effect or functionality as the whole protein it is derived from.

Variants of proteins: "Variants" of proteins or peptides as defined in the context of the present invention 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 biological function or specific activity compared to the full-length native protein, e.g. its specific antigenic property. "Variants" of proteins or peptides as defined in the context of the present invention may comprise conservative amino acid substitution(s) compared to their native, i.e. non-mutated physiological, sequence. Those amino acid sequences as well as their encoding nucleotide sequences in particular fall under the term variants as defined herein. 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 (e.g. serine (threonine) by threonine (serine) or leucine (isoleucine) by isoleucine (leucine)). 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).

A "variant" of a protein or peptide may have at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% amino acid identity over a stretch of 10, 20, 30, 50, 75 or 100 amino acids of such protein or peptide.

Furthermore, variants of proteins or peptides as defined herein, which may be encoded by a nucleic acid molecule, may also comprise those sequences, wherein nucleotides of the encoding nucleic acid sequence are exchanged according to the degeneration of the genetic code, without leading to an alteration of the respective amino acid sequence of the protein or peptide, i.e. the amino acid sequence or at least part thereof may not differ from the original sequence within the above meaning.

Preferably, a variant of a protein comprises a functional variant of the protein, which means that the variant exerts the same effect or functionality as the protein it is derived from.

Identity of a sequence: In order to determine the percentage to which two sequences are identical, e.g. nucleic acid sequences or amino acid sequences as defined herein, preferably the amino acid sequences encoded by a nucleic acid sequence of the polymeric carrier as defined herein or the amino acid sequences themselves, the sequences can be aligned in order to be subsequently compared to one another. Therefore, e.g. a position of a first sequence may be compared with the corresponding position of the second sequence. If a position in the first sequence is occupied by the same component (residue) as is the case at a position in the second sequence, the two sequences are identical at this position. If this is not the case, the sequences differ at this position. If insertions occur in the second sequence in comparison to the first sequence, gaps can be inserted into the first sequence to allow a further alignment. If deletions occur in the second sequence in comparison to the first sequence, gaps can be inserted into the second sequence to allow a further alignment. The percentage to which two sequences are identical is then a function of the number of identical positions divided by the total number of positions including those positions which are only occupied in one sequence. 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.

Monocistronic mRNA: A monocistronic mRNA may typically be an mRNA, that comprises only one coding sequence (open reading frame). A coding sequence/open reading frame in this context is a sequence of several nucleotide triplets (codons) that can be translated into a peptide or protein.

Nucleic acid: The term nucleic acid means any DNA or RNA molecule and is used synonymous with polynucleotide. Wherever herein reference is made to a nucleic acid or nucleic acid sequence encoding a particular protein and/or peptide, said nucleic acid or nucleic acid sequence, respectively, preferably also comprises regulatory sequences allowing in a suitable host, e.g. a human being, its expression, i.e. transcription and/or translation of the nucleic acid sequence encoding the particular protein or peptide.

Peptide: A peptide is a polymer of amino acid monomers. Usually the monomers are linked by peptide bonds. The term "peptide" does not limit the length of the polymer chain of amino acids. In some embodiments of the present invention a peptide may for example contain less than 50 monomer units. Longer peptides are also called polypeptides, typically having 50 to 600 monomeric units, more specifically 50 to 300 monomeric units.

Pharmaceutically effective amount: A pharmaceutically effective amount in the context of the invention is typically understood to be an amount that is sufficient to induce an immune response or to trigger the desired therapeutical effect.

Protein: A protein typically consists of one or more peptides and/or polypeptides folded into 3-dimensional form, facilitating a biological function.

Poly(C) sequence: A poly(C) sequence is typically a long sequence of cytosine nucleotides, typically about 10 to about 200 cytosine nucleotides, preferably about 10 to about 100 cytosine nucleotides, more preferably about 10 to about 70 cytosine nucleotides or even more, preferably about 20 to about 50, or even about 20 to about 30 cytosine nucleotides. A poly(C) sequence may preferably be located 3' of the coding region comprised by a nucleic acid.

Poly(A) tail: A poly(A) tail also called "3'-poly(A) tail" or "Poly(A) sequence" is typically a long homopolymeric sequence of adenosine nucleotides of up to about 400 adenosine nucleotides, e.g.

from about 25 to about 400, preferably from about 50 to about 400, more preferably from about 50 to about 300, even more preferably from about 50 to about 250, most preferably from about 60 to about 250 adenosine nucleotides, added to the 3' end of an mRNA. In the context of the present invention, the poly(A) tail of an mRNA is preferably derived from a DNA template by RNA *in vitro* transcription. Alternatively, the poly(A) sequence may also be obtained *in vitro* by common methods of chemical synthesis without being necessarily transcribed from a DNA-progenitor. Moreover, poly(A) sequences, or poly(A) tails may be generated by enzymatic polyadenylation of the RNA.

Stabilized nucleic acid: A stabilized nucleic acid, typically, exhibits a modification increasing resistance to *in vivo* degradation (e.g. degradation by an exo- or endo-nuclease) and/or *ex vivo* degradation (e.g. by the manufacturing process prior to vaccine administration, e.g. in the course of the preparation of the vaccine solution to be administered). Stabilization of RNA can, e.g., be achieved by providing a 5'-CAP-Structure, a poly(A) tail, or any other UTR-modification. It can also be achieved by backbone-modification or modification of the G/C-content or the C-content of the nucleic acid. Various other methods are known in the art and conceivable in the context of the invention.

Carrier/polymeric carrier: A carrier in the context of the invention may typically be a compound that facilitates transport and/or complexation of another compound. Said carrier may form a complex with said other compound. A polymeric carrier is a carrier that is formed of a polymer.

Cationic component: The term "cationic component" typically refers to a charged molecule, which is positively charged (cation) at a pH value of typically about 1 to 9, preferably of a pH value of or below 9 (e.g. 5 to 9), of or below 8 (e.g. 5 to 8), of or below 7 (e.g. 5 to 7), most preferably at physiological pH values, e.g. about 7.3 to 7.4. Accordingly, a cationic peptide, protein or polymer according to the present invention is positively charged under physiological conditions, particularly under physiological salt conditions of the cell *in vivo*. A cationic peptide or protein preferably contains a larger number of cationic amino acids, e.g. a larger number of Arg, His, Lys or Orn than other amino acid residues (in particular more cationic amino acids than anionic amino acid residues like Asp or Glu) or contains blocks predominantly formed by cationic amino acid residues. The definition "cationic" may also refer to "polycationic" components.

Vehicle: A vehicle is an agent, e.g. a carrier, that may typically be used within a pharmaceutical composition or vaccine for facilitating administering of the components of the pharmaceutical composition or vaccine to an individual.

3'-untranslated region (3'-UTR): A 3'-UTR is typically the part of an mRNA which is located between the protein coding region (i.e. the open reading frame) and the poly(A) sequence of the mRNA. A 3'-UTR of the mRNA is not translated into an amino acid sequence. The 3'-UTR sequence is generally encoded by the gene which is transcribed into the respective mRNA during the gene expression process. The genomic sequence is first transcribed into pre-mature mRNA, which comprises optional introns. The pre-mature mRNA is then further processed into mature mRNA in a maturation process. This maturation process comprises the steps of 5'-capping, splicing the pre-mature mRNA to excise optional introns and modifications of the 3'-end, such as polyadenylation of the 3'-end of the pre-mature mRNA and optional endo- or exonuclease cleavages etc. In the context of the present invention, a 3'-UTR corresponds to the sequence of a mature mRNA which is located 3' to the stop codon of the protein coding region, preferably immediately 3' to the stop codon of the protein coding region, and which extends to the 5'-side of the poly(A) sequence, preferably to the nucleotide immediately 5' to the poly(A) sequence. The term "corresponds to" means that the 3'-UTR sequence may be an RNA sequence, such as in the mRNA sequence used for defining the 3'-UTR sequence, or a DNA sequence which corresponds to such RNA sequence. In the context of the present invention, the term "a 3'-UTR of a gene", such as "a 3'-UTR of an albumin gene", is the sequence which corresponds to the 3'-UTR of the mature mRNA derived from this gene, i.e. the mRNA obtained by transcription of the gene and maturation of the pre-mature mRNA. The term "3'-UTR of a gene" encompasses the DNA sequence and the RNA sequence of the 3'-UTR.

5'-untranslated region (5'-UTR): A 5'-UTR is typically understood to be a particular section of messenger RNA (mRNA). It is located 5' of the open reading frame of the mRNA. Typically, the 5'-UTR starts with the transcriptional start site and ends one nucleotide before the start codon of the open reading frame. The 5'-UTR may comprise elements for controlling gene expression, also called regulatory elements. Such regulatory elements may be, for example, ribosomal binding sites or a 5'-Terminal Oligopyrimidine Tract. The 5'-UTR may be posttranscriptionally modified, for example

by addition of a 5'-CAP. In the context of the present invention, a 5'UTR corresponds to the sequence of a mature mRNA which is located between the 5'-CAP and the start codon. Preferably, the 5'-UTR corresponds to the sequence which extends from a nucleotide located 3' to the 5'-CAP, preferably from the nucleotide located immediately 3' to the 5'-CAP, to a nucleotide located 5' to the start codon of the protein coding region, preferably to the nucleotide located immediately 5' to the start codon of the protein coding region. The nucleotide located immediately 3' to the 5'-CAP of a mature mRNA typically corresponds to the transcriptional start site. The term "corresponds to" means that the 5'-UTR sequence may be an RNA sequence, such as in the mRNA sequence used for defining the 5'-UTR sequence, or a DNA sequence which corresponds to such RNA sequence. In the context of the present invention, the term "a 5'-UTR of a gene", such as "a 5'-UTR of a TOP gene", is the sequence which corresponds to the 5'-UTR of the mature mRNA derived from this gene, i.e. the mRNA obtained by transcription of the gene and maturation of the pre-mature mRNA. The term "5'-UTR of a gene" encompasses the DNA sequence and the RNA sequence of the 5'-UTR.

5' Terminal Oligopyrimidine Tract (TOP): The 5' terminal oligopyrimidine tract (TOP) is typically a stretch of pyrimidine nucleotides located at the 5' terminal region of a nucleic acid molecule, such as the 5' terminal region of certain mRNA molecules or the 5' terminal region of a functional entity, e.g. the transcribed region, of certain genes. The sequence starts with a cytidine, which usually corresponds to the transcriptional start site, and is followed by a stretch of usually about 3 to 30 pyrimidine nucleotides. For example, the TOP may comprise 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or even more nucleotides. The pyrimidine stretch and thus the 5' TOP ends one nucleotide 5' to the first purine nucleotide located downstream of the TOP. mRNA that contains a 5' terminal oligopyrimidine tract is often referred to as TOP mRNA. Accordingly, genes that provide such messenger RNAs are referred to as TOP genes. TOP sequences have, for example, been found in genes and mRNAs encoding peptide elongation factors and ribosomal proteins.

TOP motif: In the context of the present invention, a TOP motif is a nucleic acid sequence which corresponds to a 5' TOP as defined above. Thus, a TOP motif in the context of the present invention is preferably a stretch of pyrimidine nucleotides having a length of 3-30 nucleotides. Preferably, the TOP-motif consists of at least 3 pyrimidine nucleotides, preferably at least 4 pyrimidine nucleotides, preferably at least 5 pyrimidine nucleotides, more preferably at least 6 nucleotides, more

preferably at least 7 nucleotides, most preferably at least 8 pyrimidine nucleotides, wherein the stretch of pyrimidine nucleotides preferably starts at its 5' end with a cytosine nucleotide. In TOP genes and TOP mRNAs, the TOP-motif preferably starts at its 5' end with the transcriptional start site and ends one nucleotide 5' to the first purine residue in said gene or mRNA. A TOP motif in the sense of the present invention is preferably located at the 5'end of a sequence which represents a 5'-UTR or at the 5' end of a sequence which codes for a 5'-UTR. Thus, preferably, a stretch of 3 or more pyrimidine nucleotides is called "TOP motif" in the sense of the present invention if this stretch is located at the 5' end of a respective sequence, such as the inventive mRNA, the 5'-UTR element of the inventive mRNA, or the nucleic acid sequence which is derived from the 5'-UTR of a TOP gene as described herein. In other words, a stretch of 3 or more pyrimidine nucleotides which is not located at the 5'-end of a 5'-UTR or a 5'-UTR element but anywhere within a 5'-UTR or a 5'-UTR element is preferably not referred to as "TOP motif".

TOP gene: TOP genes are typically characterised by the presence of a 5' terminal oligopyrimidine tract. Furthermore, most TOP genes are characterized by a growth-associated translational regulation. However, also TOP genes with a tissue specific translational regulation are known. As defined above, the 5'-UTR of a TOP gene corresponds to the sequence of a 5'-UTR of a mature mRNA derived from a TOP gene, which preferably extends from the nucleotide located 3' to the 5'-CAP to the nucleotide located 5' to the start codon. A 5'-UTR of a TOP gene typically does not comprise any start codons, preferably no upstream AUGs (uAUGs) or upstream open reading frames (uORFs). Therein, upstream AUGs and upstream open reading frames are typically understood to be AUGs and open reading frames that occur 5' of the start codon (AUG) of the open reading frame that should be translated. The 5'-UTRs of TOP genes are generally rather short. The lengths of 5'-UTRs of TOP genes may vary between 20 nucleotides up to 500 nucleotides, and are typically less than about 200 nucleotides, preferably less than about 150 nucleotides, more preferably less than about 100 nucleotides. Exemplary 5'-UTRs of TOP genes in the sense of the present invention are the nucleic acid sequences extending from the nucleotide at position 5 to the nucleotide located immediately 5' to the start codon (e.g. the ATG) in the sequences according to SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the international patent application WO2013/143700 or homologs or variants thereof, whose disclosure is incorporated herewith by reference. In this context a particularly preferred fragment of a 5'UTR of

a TOP gene is a 5'-UTR of a TOP gene lacking the 5' TOP motif. The term '5'UTR of a TOP gene' preferably refers to the 5'-UTR of a naturally occurring TOP gene.

Chemical synthesis of RNA: Chemical synthesis of relatively short fragments of oligonucleotides with defined chemical structure provides a rapid and inexpensive access to custom-made oligonucleotides of any desired sequence. Whereas enzymes synthesize DNA and RNA only in the 5' to 3' direction, chemical oligonucleotide synthesis does not have this limitation, although it is most often carried out in the opposite, i.e. the 3' to 5' direction. Currently, the process is implemented as solid-phase synthesis using the phosphoramidite method and phosphoramidite building blocks derived from protected nucleosides (A, C, G, and U), or chemically modified nucleosides.

To obtain the desired oligonucleotide, the building blocks are sequentially coupled to the growing oligonucleotide chain on a solid phase in the order required by the sequence of the product in a fully automated process. Upon the completion of the chain assembly, the product is released from the solid phase to the solution, deprotected, and collected. The occurrence of side reactions sets practical limits for the length of synthetic oligonucleotides (up to about 200 nucleotide residues), because the number of errors increases with the length of the oligonucleotide being synthesized. Products are often isolated by HPLC to obtain the desired oligonucleotides in high purity.

Chemically synthesized oligonucleotides find a variety of applications in molecular biology and medicine. They are most commonly used as antisense oligonucleotides, small interfering RNA, primers for DNA sequencing and amplification, probes for detecting complementary DNA or RNA via molecular hybridization, tools for the targeted introduction of mutations and restriction sites, and for the synthesis of artificial genes.

RNA *In vitro* transcription: The terms "RNA *in vitro* transcription" or "*in vitro* transcription" relate to a process wherein RNA is synthesized in a cell-free system (*in vitro*). DNA, particularly plasmid DNA, is used as template for the generation of RNA transcripts. RNA may be obtained by DNA-dependent *in vitro* transcription of an appropriate DNA template, which according to the present invention is preferably a linearized plasmid DNA template. The promoter for controlling *in vitro* transcription can be any promoter for any DNA-dependent RNA polymerase. Particular examples of DNA-dependent RNA polymerases are the T7, T3, and SP6 RNA polymerases. A DNA template for *in vitro*

RNA transcription may be obtained by cloning of a nucleic acid, in particular cDNA corresponding to the respective RNA to be *in vitro* transcribed, and introducing it into an appropriate vector for *in vitro* transcription, for example into plasmid DNA. In a preferred embodiment of the present invention the DNA template is linearized with a suitable restriction enzyme, before it is transcribed *in vitro*. The cDNA may be obtained by reverse transcription of mRNA or chemical synthesis. Moreover, the DNA template for *in vitro* RNA synthesis may also be obtained by gene synthesis.

Methods for *in vitro* transcription are known in the art (see, e.g., Geall et al. (2013) Semin. Immunol. 25(2): 152–159; Brunelle et al. (2013) Methods Enzymol. 530:101-14). Reagents used in said method typically include:

1) a linearized DNA template with a promoter sequence that has a high binding affinity for its respective RNA polymerase such as bacteriophage-encoded RNA polymerases;

2) ribonucleoside triphosphates (NTPs) for the four bases (adenine, cytosine, guanine and uracil);

3) optionally a cap analogue as defined above (e.g. m7G(5')ppp(5')G (m7G));

4) a DNA-dependent RNA polymerase capable of binding to the promoter sequence within the linearized DNA template (e.g. T7, T3 or SP6 RNA polymerase);

5) optionally a ribonuclease (RNase) inhibitor to inactivate any contaminating RNase;

6) optionally a pyrophosphatase to degrade pyrophosphate, which may inhibit transcription;

7) $MgCl_2$, which supplies Mg^{2+} ions as a co-factor for the polymerase;

8) a buffer to maintain a suitable pH value, which can also contain antioxidants (e.g. DTT), and/or polyamines such as spermidine at optimal concentrations.

RNA, mRNA: RNA is the usual abbreviation for ribonucleic acid. It is a nucleic acid molecule, i.e. a polymer consisting of nucleotide monomers. These nucleotides are usually adenosine monophosphate (AMP), uridine monophosphate (UMP), guanosine monophosphate (GMP) and cytidine monophosphate (CMP) monomers or analogues thereof, which are connected to each other along a so-called backbone. The backbone is formed by phosphodiester bonds between the sugar, i.e. ribose, of a first and a phosphate moiety of a second, adjacent monomer. The specific order of the monomers, i.e. the order of the bases linked to the sugar/phosphate-backbone, is called the RNA sequence. Usually RNA may be obtainable by transcription of a DNA sequence, e.g., inside a cell. In eukaryotic cells, transcription is typically performed inside the nucleus or the mitochondria. *In vivo*, transcription of DNA usually results in the so-called premature RNA (also

called pre-mRNA, precursor mRNA or heterogeneous nuclear RNA) which has to be processed into so-called messenger RNA, usually abbreviated as mRNA. Processing of the premature RNA, e.g. in eukaryotic organisms, comprises a variety of different posttranscriptional modifications such as splicing, 5'-capping, polyadenylation, export from the nucleus or the mitochondria and the like. The sum of these processes is also called maturation of RNA. The mature messenger RNA usually provides the nucleotide sequence that may be translated into an amino acid sequence of a particular peptide or protein. Typically, a mature mRNA comprises a 5'-cap, optionally a 5'UTR, an open reading frame, optionally a 3'UTR and a poly(A) tail.

In addition to messenger RNA, several non-coding types of RNA exist which may be involved in regulation of transcription and/or translation, and immunostimulation. Within the present invention the term "RNA" further encompasses any type of single stranded (ssRNA) or double stranded RNA (dsRNA) molecule known in the art, such as viral RNA, retroviral RNA and replicon RNA, small interfering RNA (siRNA), antisense RNA (asRNA), circular RNA (circRNA), ribozymes, aptamers, riboswitches, immunostimulating/immunostimulatory RNA, transfer RNA (tRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), microRNA (miRNA), and Piwi-interacting RNA (piRNA).

Fragment of a nucleic acid sequence, particularly an RNA: A fragment of a nucleic acid sequence consists of a continuous stretch of nucleotides corresponding to a continuous stretch of nucleotides in the full-length nucleic acid sequence which is the basis for the nucleic acid sequence of the fragment, which represents at least 20%, preferably at least 30%, more preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, even more preferably at least 70%, even more preferably at least 80%, and most preferably at least 90% of the full-length nucleic acid sequence. Such a fragment, in the sense of the present invention, is preferably a functional fragment of the full-length nucleic acid sequence.

Variant of a nucleic acid sequence, particularly anRNA: A variant of a nucleic acid sequence refers to a variant of nucleic acid sequences which forms the basis of a nucleic acid sequence. For example, a variant nucleic acid sequence may exhibit one or more nucleotide deletions, insertions, additions and/or substitutions compared to the nucleic acid sequence from which the variant is derived. Preferably, a variant of a nucleic acid sequence is at least 40%, preferably at least 50%,

more preferably at least 60%, more preferably at least 70%, even more preferably at least 80%, even more preferably at least 90%, most preferably at least 95% identical to the nucleic acid sequence the variant is derived from. Preferably, the variant is a functional variant. A "variant" of a nucleic acid sequence may have at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% nucleotide identity over a stretch of 10, 20, 30, 50, 75 or 100 nucleotide of such nucleic acid sequence.

Intratumoral administration/application: The term „intratumoral administration/application" refers to the direct delivery of a pharmaceutical composition into or adjacent to a tumor or cancer and/or immediate vicinity of a tumor or cancer. Multiple injections into separate regions of the tumor or cancer are also included. Furthermore, intratumoral administration/application includes delivery of a pharmaceutical composition into one or more metastases.

Methods for intratumoral delivery of drugs are known in the art (Brincker, 1993. Crit. Rev. Oncol. Hematol. 15(2):91-8; Celikoglu et al., 2008. Cancer Therapy 6, 545-552). For example, the pharmaceutical composition can be administered by conventional needle injection, needle-free jet injection or electroporation or combinations thereof into the tumor or cancer tissue. The pharmaceutical composition can be injected directly into the tumor or cancer (tissue) with great precision using computer tomograpy, ultrasound, gamma camera imaging, positron emission tomography, or magnetic resonance tumor imaging. Further procedures are selected from the group including, but not limited to, direct intratumoral injection by endoscopy, bronchoscopy, cystoscopy, colonoscopy, laparoscope and catheterization.

Decoy receptors: Decoy receptors recognize certain growth factors or cytokines with high affinity and specificity, but are structurally incapable of signaling or presenting the agonist to signaling receptor complexes. They act as a molecular trap for the agonist and for signaling receptor components. A decoy receptor, or sink receptor, is a receptor that binds a ligand, inhibiting it from binding to its normal receptor. For instance, the receptor VEGFR-1 can prevent vascular endothelial growth factor (VEGF) from binding to the VEGFR-2.

Dominant negative receptors: Dominant negative receptors are variants of the particular receptor comprising dominant-negative (DN) mutations as leading to mutant polypeptides that disrupt the activity of the wild-type receptor when overexpressed.

Detailed description of the invention

The RNA containing composition according to the invention comprises at least one RNA and is particularly provided for use in the treatment or prophylaxis of tumor and/or cancer diseases, wherein the RNA containing composition is preferably applied/administered intratumorally. It is especially preferred that the RNA containing composition is injected directly into tumor tissue. Alternatively, it is especially preferred that the RNA containing composition is injected adjacent to or in close proximity to a tumor tissue and/or metastasis.

It has been found by the inventors that intratumoral application respectively administration of the RNA containing composition according to the invention is capable of effectively treating tumor and/or cancer diseases and related disorders. It has been shown that intratumoral application is surprisingly effective in decreasing tumor size. Moreover the application of the RNA containing composition according to the invention was able to increase survival in animal models.

The at least one RNA of the RNA containing composition may be selected from the group consisting of chemically modified or unmodified RNA, single-stranded or double-stranded RNA, coding or non-coding RNA, mRNA, oligoribonucleotide, viral RNA, retroviral RNA, replicon RNA, tRNA, rRNA, immunostimulatory RNA, microRNA, siRNA, small nuclear RNA (snRNA), small-hairpin (sh) RNA riboswitch, RNA aptamer, RNA decoy, antisense RNA, a ribozyme, or any combination thereof.

In specific embodiments the at least one RNA of the RNA containing composition is selected from a coding RNA or a non-coding RNA.

Coding RNA:

According to a preferred embodiment of the invention the at least one RNA of the RNA containing composition comprises at least one coding region encoding at least one peptide or protein.

Preferably, the coding RNA is selected from the group consisting of mRNA, viral RNA, retroviral RNA, and replicon RNA.

In preferred embodiments of the invention the at least one RNA of the RNA containing composition codes for at least one cytokine and/or for at least one chemokine and/or for at least one suicide gene product, and/or at least one immunogenic protein or peptide and/or for at least one cell death/apoptosis inducer and/or for at least one angiogenesis inhibitor and/or for at least one heat shock protein and/or for at least one tumor antigen and/or for at least one β-catenin inhibitor

and/or for at least one activator of the STING (stimulator of interferon genes) pathway and/or at least one checkpoint modulator and/or at least one antibody, and/or at least one dominant negative receptor, and/or at least one decoy receptor, and/or at least one inhibitor of myeloid derived suppressor cells (MDSCs), and/or at least one IDO pathway inhibitor, and/or at least one protein or peptide that bind inhibitors of apoptosis, or fragments or variants thereof as will be outlined in more detail below.

1. Cytokines

In a preferred embodiment of the inventive RNA containing composition the RNA comprises at least one coding region that codes for at least one cytokine, or a fragment or variant thereof.

Preferably the cytokine is an interleukin (IL). One or more interleukins may be chosen e.g. from the following list: IL-1α, IL-1β, IL-1ra (antagonist), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10; IL-11, IL-12, IL-13, IL14, IL-15, IL-16, IL-17A, IL-17B, EL-17C, IL-17D, IL-17E, IL-17F, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28A/B, IL-29, IL-30, IL-31, IL-32, IL-33, IL-35. Moreover the cytokine may be one or more cytokines chosen from the TNF family, e.g. chosen from the following list: TNF, especially TNFα, LTα, LTβ, LIGHT, TWEAK, APRIL, BAFF, TL1A, GITRL, OX40L, CD40L (CD154), FASL, CD27L, CD30L, 4-1BBL, TRAIL, RANK ligand. Further examples of preferred cytokines may be chosen from the following list: FLT3 ligand, G-CSF, GM-CSF, IFNα/β/ω, IFNγ, LIF, M-CSF, MIF, OSM, Stem Cell Factor, TGFβ1, TGFβ2, TGFβ3, TSLP ligand.

Particularly preferred are cytokines chosen from the following list: IL-12, IL-15, IL-2, IFNγ, TNFα, IL-18, IFNα, IL-1β, IL-32, IL-7, IL-21, IL-8, GM-CSF.

In an especially preferred embodiment of the invention the RNA of the inventive composition codes for Interleukin-12 or CD40L. It has been shown by the inventors, that mRNA coding for this cytokines is especially effective when applied in the inventive approach. Particularly preferred are RNA sequences according to SEQ ID Nos. 1, 3, 4194, 4195, 4196, 4197, 4198, 4199, 4200 encoding IL-12. Furthermore RNA sequences according to SEQ ID Nos. 3898, 3899, 3900, 3901, 3902, 3903, 3904, 10073, encoding CD40L are particularly preferred.

According to preferred embodiments in the context of the present invention cytokines may be selected from any cytokine selected from the group consisting of 4-1BBL; Apo2L/TRAIL; APRIL; BAFF; CD27L; CD30L; CD40L_(CD154); CXCL8; EL-17C; FasL; FLT3_ligand; G-CSF; GITRL; GM-CSF;

IFNalpha; IFNB; IFNG; IFNomega; IL-1_alpha; IL-1_beta; IL-10; IL-11; IL-12; IL-12A; IL-13; IL-14; IL-15; IL-16; IL-17A; IL-17B; IL-17D; IL-17F; IL-18; IL-19; IL-1ra_(antagonist); IL-2; IL-20; IL-21; IL-22; IL-23; IL-24; IL-25; IL-26; IL-27A; IL-27B; IL-28A; IL-28B; IL-29; IL-3; IL-31; IL-32; IL-33; IL-37; IL-4; IL-5; IL-6; IL-7; IL-9; LIF; LIGHT; LTalpha; LTbeta; M-CSF; MIF; OSM; OX40L; RANK_ligand; Stem_Cell_Factor; TGFbeta1; TGFbeta2; TGFbeta3; TL1A; TNF; TWEAK, preferably as disclosed in Table 1. Particularly preferred in this context are the RNA sequences encoding a cytokine according to Table 1.

Table 1: Cytokines:

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
4-1BBL	UniProtKB: P41273	3849	3850	3851, 3852, 3853, 3854, 3855, 3856
APRIL	UniProtKB: O75888	3857	3858	3859, 3860, 3861, 3862, 3863, 3864
BAFF	UniProtKB: Q5H8V1	3865	3866	3867, 3868, 3869, 3870, 3871, 3872
BAFF	UniProtKB: Q9Y275	3873	3874	3875, 3876, 3877, 3878, 3879, 3880
CD27L	UniProtKB: P32970	3881	3882	3883, 3884, 3885, 3886, 3887, 3888
CD30L	UniProtKB: P32971	3889	3890	3891, 3892, 3893, 3894, 3895, 3896
CD40L_(CD154)	UniProtKB: P29965	3897	3898	3899, 3900, 3901, 3902, 3903, 3904
EL-17C	UniProtKB: Q9P0M4	3905	3906	3907, 3908, 3909, 3910, 3911, 3912
FLT3_ligand	Genbank: AAA90950.1	3913	3914	3915, 3916, 3917, 3918, 3919, 3920
FLT3_ligand	UniProtKB: P49771	3921	3922	3923, 3924, 3925, 3926, 3927, 3928
G-CSF	UniProtKB: P09919	3929	3930	3931, 3932, 3933, 3934, 3935, 3936
GITRL	UniProtKB: Q9UNG2	3937	3938	3939, 3940, 3941, 3942, 3943, 3944
GM-CSF	UniProtKB: P04141	3945	3946	3947, 3948, 3949, 3950, 3951, 3952
IFNalpha	UniProtKB: G9JKF1	3953	3954	3955, 3956, 3957, 3958, 3959, 3960
IFNalpha	UniProtKB: P01562	3961	3962	3963, 3964, 3965, 3966,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				3967, 3968
IFNalpha	UniProtKB: P01563	3969	3970	3971, 3972, 3973, 3974, 3975, 3976
IFNalpha	UniProtKB: P01566	3977	3978	3979, 3980, 3981, 3982, 3983, 3984
IFNalpha	UniProtKB: P01567	3985	3986	3987, 3988, 3989, 3990, 3991, 3992
IFNalpha	UniProtKB: P01568	3993	3994	3995, 3996, 3997, 3998, 3999, 4000
IFNalpha	UniProtKB: P01569	4001	4002	4003, 4004, 4005, 4006, 4007, 4008
IFNalpha	UniProtKB: P01570	4009	4010	4011, 4012, 4013, 4014, 4015, 4016
IFNalpha	UniProtKB: P01571	4017	4018	4019, 4020, 4021, 4022, 4023, 4024
IFNalpha	UniProtKB: P05013	4025	4026	4027, 4028, 4029, 4030, 4031, 4032
IFNalpha	UniProtKB: P05014	4033	4034	4035, 4036, 4037, 4038, 4039, 4040
IFNalpha	UniProtKB: P05015	4041	4042	4043, 4044, 4045, 4046, 4047, 4048
IFNalpha	UniProtKB: P32881	4049	4050	4051, 4052, 4053, 4054, 4055, 4056
IFNalpha	UniProtKB: Q14618	4057	4058	4059, 4060, 4061, 4062, 4063, 4064
IFNalpha	UniProtKB: Q86UP4	4065	4066	4067, 4068, 4069, 4070, 4071, 4072
IFNB	UniProtKB: P01574	4073	4074	4075, 4076, 4077, 4078, 4079, 4080
IFNB	UniProtKB: Q15943	4081	4082	4083, 4084, 4085, 4086, 4087, 4088
IFNG	UniProtKB: P01579	4089	4090	4091, 4092, 4093, 4094, 4095, 4096
IFNG	UniProtKB: Q14609	4097	4098	4099, 4100, 4101, 4102, 4103, 4104
IFNG	UniProtKB: Q14610	4105	4106	4107, 4108, 4109, 4110, 4111, 4112
IFNG	UniProtKB: Q14611	4113	4114	4115, 4116, 4117, 4118, 4119, 4120
IFNG	UniProtKB: Q14612	4121	4122	4123, 4124, 4125, 4126, 4127, 4128

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
IFNG	UniProtKB: Q14613	4129	4130	4131, 4132, 4133, 4134, 4135, 4136
IFNG	UniProtKB: Q14614	4137	4138	4139, 4140, 4141, 4142, 4143, 4144
IFNG	UniProtKB: Q14615	4145	4146	4147, 4148, 4149, 4150, 4151, 4152
IFNG	UniProtKB: Q8NHY9	4153	4154	4155, 4156, 4157, 4158, 4159, 4160
IFNomega	UniProtKB: P05000	4161	4162	4163, 4164, 4165, 4166, 4167, 4168
IL-10	UniProtKB: P22301	4169	4170	4171, 4172, 4173, 4174, 4175, 4176
IL-11	UniProtKB: P20809	4177	4178	4179, 4180, 4181, 4182, 4183, 4184
IL-12A	UniProtKB: P29459	4185	4186	4187, 4188, 4189, 4190, 4191, 4192
IL-12	UniProtKB: P29460	4193	4194	4195, 4196, 4197, 4198, 4199, 4200
IL-13	UniProtKB: P35225	4201	4202	4203, 4204, 4205, 4206, 4207, 4208
IL-14	UniProtKB: P40222	4209	4210	4211, 4212, 4213, 4214, 4215, 4216
IL-15	UniProtKB: P40933	4217	4218	4219, 4220, 4221, 4222, 4223, 4224
IL-16	UniProtKB: Q14005	4225	4226	4227, 4228, 4229, 4230, 4231, 4232
IL-17A	UniProtKB: Q16552	4233	4234	4235, 4236, 4237, 4238, 4239, 4240
IL-17B	UniProtKB: Q9NRM6	4241	4242	4243, 4244, 4245, 4246, 4247, 4248
IL-17B	UniProtKB: Q9UHF5	4249	4250	4251, 4252, 4253, 4254, 4255, 4256
IL-17D	UniProtKB: Q8TAD2	4257	4258	4259, 4260, 4261, 4262, 4263, 4264
IL-17F	UniProtKB: F1JZ09	4265	4266	4267, 4268, 4269, 4270, 4271, 4272
IL-17F	UniProtKB: Q96PD4	4273	4274	4275, 4276, 4277, 4278, 4279, 4280
IL-18	UniProtKB: A0A024R3E0	4281	4282	4283, 4284, 4285, 4286, 4287, 4288
IL-18	UniProtKB: B0YJ28	4289	4290	4291, 4292, 4293, 4294,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				4295, 4296
IL-18	UniProtKB: Q14116	4297	4298	4299, 4300, 4301, 4302, 4303, 4304
IL-19	UniProtKB: Q9UHD0	4305	4306	4307, 4308, 4309, 4310, 4311, 4312
IL-1_alpha	UniProtKB: P01583	4313	4314	4315, 4316, 4317, 4318, 4319, 4320
IL-1_beta	UniProtKB: P01584	4321	4322	4323, 4324, 4325, 4326, 4327, 4328
IL-1ra_(antagonist)	UniProtKB: P18510-2	4329	4330	4331, 4332, 4333, 4334, 4335, 4336
IL-1ra_(antagonist)	UniProtKB: P18510-3	4337	4338	4339, 4340, 4341, 4342, 4343, 4344
IL-1ra_(antagonist)	UniProtKB: P18510	4345	4346	4347, 4348, 4349, 4350, 4351, 4352
IL-20	UniProtKB: Q9NYY1	4353	4354	4355, 4356, 4357, 4358, 4359, 4360
IL-21	RefSeq: NP_001193935.1	4361	4362	4363, 4364, 4365, 4366, 4367, 4368
IL-21	RefSeq: NP_068575.1	4369	4370	4371, 4372, 4373, 4374, 4375, 4376
IL-22	UniProtKB: Q9GZX6	4377	4378	4379, 4380, 4381, 4382, 4383, 4384
IL-23	UniProtKB: Q9NPF7	4385	4386	4387, 4388, 4389, 4390, 4391, 4392
IL-24	UniProtKB: Q13007	4393	4394	4395, 4396, 4397, 4398, 4399, 4400
IL-24	UniProtKB: Q2YHE6	4401	4402	4403, 4404, 4405, 4406, 4407, 4408
IL-25	UniProtKB: Q969H8	4409	4410	4411, 4412, 4413, 4414, 4415, 4416
IL-25	UniProtKB: Q9H293	4417	4418	4419, 4420, 4421, 4422, 4423, 4424
IL-26	UniProtKB: Q9NPH9	4425	4426	4427, 4428, 4429, 4430, 4431, 4432
IL-27A	UniProtKB: Q8NEV9	4433	4434	4435, 4436, 4437, 4438, 4439, 4440
IL-27B	UniProtKB: Q14213	4441	4442	4443, 4444, 4445, 4446, 4447, 4448
IL-28A	UniProtKB: Q8IZJ0	4449	4450	4451, 4452, 4453, 4454, 4455, 4456

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
IL-28B	UniProtKB: Q8IZI9	4457	4458	4459, 4460, 4461, 4462, 4463, 4464
IL-29	UniProtKB: Q8IU54	4465	4466	4467, 4468, 4469, 4470, 4471, 4472
IL-2	UniProtKB: P60568	4473	4474	4475, 4476, 4477, 4478, 4479, 4480
IL-2	UniProtKB: Q0GK43	4481	4482	4483, 4484, 4485, 4486, 4487, 4488
IL-2	UniProtKB: Q13169	4489	4490	4491, 4492, 4493, 4494, 4495, 4496
IL-2	UniProtKB: Q6NZ91	4497	4498	4499, 4500, 4501, 4502, 4503, 4504
IL-2	UniProtKB: Q6NZ93	4505	4506	4507, 4508, 4509, 4510, 4511, 4512
IL-31	UniProtKB: Q6EBC2	4513	4514	4515, 4516, 4517, 4518, 4519, 4520
IL-32	UniProtKB: P24001	4521	4522	4523, 4524, 4525, 4526, 4527, 4528
IL-33	UniProtKB: O95760	4529	4530	4531, 4532, 4533, 4534, 4535, 4536
IL-37	UniProtKB: Q9NZH6	4537	4538	4539, 4540, 4541, 4542, 4543, 4544
IL-3	UniProtKB: P08700	4545	4546	4547, 4548, 4549, 4550, 4551, 4552
IL-3	UniProtKB: Q6NZ78	4553	4554	4555, 4556, 4557, 4558, 4559, 4560
IL-3	UniProtKB: Q6NZ79	4561	4562	4563, 4564, 4565, 4566, 4567, 4568
IL-4	UniProtKB: P05112-2	4569	4570	4571, 4572, 4573, 4574, 4575, 4576
IL-4	UniProtKB: P05112	4577	4578	4579, 4580, 4581, 4582, 4583, 4584
IL-5	UniProtKB: P05113	4585	4586	4587, 4588, 4589, 4590, 4591, 4592
IL-6	UniProtKB: P05231	4593	4594	4595, 4596, 4597, 4598, 4599, 4600
IL-7	UniProtKB: A8K673	4601	4602	4603, 4604, 4605, 4606, 4607, 4608
IL-7	UniProtKB: P13232	4609	4610	4611, 4612, 4613, 4614, 4615, 4616
IL-9	UniProtKB: P15248	4617	4618	4619, 4620, 4621, 4622,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				4623, 4624
LIF	UniProtKB: P15018	4625	4626	4627, 4628, 4629, 4630, 4631, 4632
LIGHT	UniProtKB: O43557	4633	4634	4635, 4636, 4637, 4638, 4639, 4640
LTalpha	UniProtKB: B4DVZ8	4641	4642	4643, 4644, 4645, 4646, 4647, 4648
LTalpha	UniProtKB: P01374	4649	4650	4651, 4652, 4653, 4654, 4655, 4656
LTalpha	UniProtKB: P09960	4657	4658	4659, 4660, 4661, 4662, 4663, 4664
LTalpha	UniProtKB: Q5ST95	4665	4666	4667, 4668, 4669, 4670, 4671, 4672
LTalpha	UniProtKB: Q5STV3	4673	4674	4675, 4676, 4677, 4678, 4679, 4680
LTalpha	UniProtKB: Q6FG55	4681	4682	4683, 4684, 4685, 4686, 4687, 4688
LTbeta	UniProtKB: Q06643	4689	4690	4691, 4692, 4693, 4694, 4695, 4696
LTbeta	UniProtKB: Q5STB2	4697	4698	4699, 4700, 4701, 4702, 4703, 4704
M-CSF	UniProtKB: P09603	4705	4706	4707, 4708, 4709, 4710, 4711, 4712
MIF	UniProtKB: A6MUU8	4713	4714	4715, 4716, 4717, 4718, 4719, 4720
MIF	UniProtKB: P14174	4721	4722	4723, 4724, 4725, 4726, 4727, 4728
OSM	UniProtKB: P13725	4729	4730	4731, 4732, 4733, 4734, 4735, 4736
OX40L	UniProtKB: P23510	4737	4738	4739, 4740, 4741, 4742, 4743, 4744
OX40L	UniProtKB: P43489	4745	4746	4747, 4748, 4749, 4750, 4751, 4752
RANK_ligand	UniProtKB: O14788	4753	4754	4755, 4756, 4757, 4758, 4759, 4760
Stem_Cell_Factor	UniProtKB: P21583-2	4761	4762	4763, 4764, 4765, 4766, 4767, 4768
Stem_Cell_Factor	UniProtKB: P21583	4769	4770	4771, 4772, 4773, 4774, 4775, 4776
TGFbeta1	UniProtKB: A0A024R0P8	4777	4778	4779, 4780, 4781, 4782, 4783, 4784

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
TGFbeta1	UniProtKB: P01137	4785	4786	4787, 4788, 4789, 4790, 4791, 4792
TGFbeta2	UniProtKB: P61812	4793	4794	4795, 4796, 4797, 4798, 4799, 4800
TGFbeta3	UniProtKB: A5YM40	4801	4802	4803, 4804, 4805, 4806, 4807, 4808
TGFbeta3	UniProtKB: P10600	4809	4810	4811, 4812, 4813, 4814, 4815, 4816
TL1A	UniProtKB: O95150	4817	4818	4819, 4820, 4821, 4822, 4823, 4824
TWEAK	UniProtKB: Q4ACW9	4825	4826	4827, 4828, 4829, 4830, 4831, 4832
CXCL8	UniProtKB: P10145	5265	5266	5267, 5268, 5269, 5270, 5271, 5272
Apo2L/TRAIL	UniProtKB: P50591	6897	6898	6899, 6900, 6901, 6902, 6903, 6904
FasL	UniProtKB: P48023	7321	7322	7323, 7324, 7325, 7326, 7327, 7328
TNF	UniProtKB: P01375	7369	7370	7371, 7372, 7373, 7374, 7375, 7376
TNF	UniProtKB: Q5STB3	7377	7378	7379, 7380, 7381, 7382, 7383, 7384

According to the present invention, in a more preferred embodiment, the inventive composition comprises at least one RNA, preferably an mRNA comprising at least one coding region encoding at least one cytokine or a fragment or variant thereof, wherein the at least one coding region comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequences according to the SEQ ID Nos as disclosed in Table 1.

2. Chemokines:

In a further preferred embodiment of the inventive RNA containing composition the RNA comprises at least one coding region that codes for at least one chemokine, or a fragment or variant thereof. Chemokines are chemotactic cytokines that control the migratory patterns and positioning of immune cells, as reviewed by Griffith et al., 2014. Annu. Rev. Immunol. 32:659-702 (PMID

24655300). Chemokine function is critical for all immune cell movement ranging from the migration required for immune cell development and homeostasis, to that required for the generation of primary and amnestic cellular and humoral immune responses, to the pathologic recruitment of immune cells in disease. Chemokines constitute the largest family of cytokines, consisting of approximately 50 endogenous chemokine ligands in humans and mice.

According to preferred embodiments in the context of the present invention chemokines may be selected from any chemokine selected from the group consisting of CCL1; CCL11; CCL12; CCL13; CCL14; CCL15; CCL16; CCL17; CCL18; CCL19; CCL2; CCL20; CCL21; CCL22; CCL24; CCL25; CCL26; CCL27; CCL28; CCL3; CCL4; CCL5; CCL6; CCL7; CCL8; CCL9; CX3CL1; CXCL1; CXCL10; CXCL11; CXCL12; CXCL13; CXCL14; CXCL15; CXCL2; CXCL3; CXCL4; CXCL5; CXCL6; CXCL7; CXCL8; CXCL9; XCL1; XCL2, preferably as disclosed in Table 2. Particularly preferred in this context are the RNA sequences encoding a chemokine according to Table 2.

Table 2: Chemokines

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	RNA Sequence SEQ ID NO:
CCL11	UniProtKB: P51671	4833	4834	4835, 4836, 4837, 4838, 4839, 4840
CCL11	UniProtKB: Q6I9T4	4841	4842	4843, 4844, 4845, 4846, 4847, 4848
CCL12	UniProtKB: Q62401	4849	4850	4851, 4852, 4853, 4854, 4855, 4856
CCL13	UniProtKB: Q99616	4857	4858	4859, 4860, 4861, 4862, 4863, 4864
CCL14	UniProtKB: Q16627	4865	4866	4867, 4868, 4869, 4870, 4871, 4872
CCL15	UniProtKB: Q16663	4873	4874	4875, 4876, 4877, 4878, 4879, 4880
CCL16	UniProtKB: O15467	4881	4882	4883, 4884, 4885, 4886, 4887, 4888
CCL17	UniProtKB: Q92583	4889	4890	4891, 4892, 4893, 4894, 4895, 4896
CCL18	UniProtKB: P55774	4897	4898	4899, 4900, 4901, 4902, 4903, 4904
CCL19	UniProtKB: Q6IBD6	4905	4906	4907, 4908, 4909, 4910, 4911, 4912

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	RNA Sequence SEQ ID NO:
CCL19	UniProtKB: Q99731	4913	4914	4915, 4916, 4917, 4918, 4919, 4920
CCL1	UniProtKB: P22362	4921	4922	4923, 4924, 4925, 4926, 4927, 4928
CCL20	UniProtKB: P78556	4929	4930	4931, 4932, 4933, 4934, 4935, 4936
CCL21	UniProtKB: O00585	4937	4938	4939, 4940, 4941, 4942, 4943, 4944
CCL22	UniProtKB: O00626	4945	4946	4947, 4948, 4949, 4950, 4951, 4952
CCL24	UniProtKB: O00175	4953	4954	4955, 4956, 4957, 4958, 4959, 4960
CCL25	UniProtKB: O15444	4961	4962	4963, 4964, 4965, 4966, 4967, 4968
CCL26	UniProtKB: Q9Y258	4969	4970	4971, 4972, 4973, 4974, 4975, 4976
CCL27	UniProtKB: Q5VZ77	4977	4978	4979, 4980, 4981, 4982, 4983, 4984
CCL28	UniProtKB: A0N0Q3	4985	4986	4987, 4988, 4989, 4990, 4991, 4992
CCL28	UniProtKB: Q9NRJ3	4993	4994	4995, 4996, 4997, 4998, 4999, 5000
CCL2	UniProtKB: P13500	5001	5002	5003, 5004, 5005, 5006, 5007, 5008
CCL3	UniProtKB: A0N0R1	5009	5010	5011, 5012, 5013, 5014, 5015, 5016
CCL3	UniProtKB: P10147	5017	5018	5019, 5020, 5021, 5022, 5023, 5024
CCL4	UniProtKB: P13236	5025	5026	5027, 5028, 5029, 5030, 5031, 5032
CCL4	UniProtKB: Q7M4M2	5033	5034	5035, 5036, 5037, 5038, 5039, 5040
CCL5	UniProtKB: D0EI67	5041	5042	5043, 5044, 5045, 5046, 5047, 5048
CCL5	UniProtKB: P13501	5049	5050	5051, 5052, 5053, 5054, 5055, 5056
CCL6	UniProtKB: P27784	5057	5058	5059, 5060, 5061, 5062, 5063, 5064
CCL7	UniProtKB: P80098	5065	5066	5067, 5068, 5069, 5070, 5071, 5072
CCL7	UniProtKB: Q7Z7Q8	5073	5074	5075, 5076, 5077, 5078, 5079,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	RNA Sequence SEQ ID NO:
				5080
CCL8	UniProtKB: H0UIC7	5081	5082	5083, 5084, 5085, 5086, 5087, 5088
CCL8	UniProtKB: P80075	5089	5090	5091, 5092, 5093, 5094, 5095, 5096
CCL9	UniProtKB: P51670	5097	5098	5099, 5100, 5101, 5102, 5103, 5104
CX3CL1	UniProtKB: A0N0N7	5105	5106	5107, 5108, 5109, 5110, 5111, 5112
CX3CL1	UniProtKB: P78423	5113	5114	5115, 5116, 5117, 5118, 5119, 5120
CX3CL1	UniProtKB: Q6I9S9	5121	5122	5123, 5124, 5125, 5126, 5127, 5128
CXCL10	UniProtKB: A0A024RDA4	5129	5130	5131, 5132, 5133, 5134, 5135, 5136
CXCL10	UniProtKB: P02778	5137	5138	5139, 5140, 5141, 5142, 5143, 5144
CXCL11	UniProtKB: O14625	5145	5146	5147, 5148, 5149, 5150, 5151, 5152
CXCL12	UniProtKB: P48061	5153	5154	5155, 5156, 5157, 5158, 5159, 5160
CXCL13	UniProtKB: L8E878	5161	5162	5163, 5164, 5165, 5166, 5167, 5168
CXCL13	UniProtKB: O43927	5169	5170	5171, 5172, 5173, 5174, 5175, 5176
CXCL14	UniProtKB: O95715	5177	5178	5179, 5180, 5181, 5182, 5183, 5184
CXCL15	UniProtKB: Q9WVL7	5185	5186	5187, 5188, 5189, 5190, 5191, 5192
CXCL1	UniProtKB: P09341	5193	5194	5195, 5196, 5197, 5198, 5199, 5200
CXCL2	UniProtKB: A0A024RDD9	5201	5202	5203, 5204, 5205, 5206, 5207, 5208
CXCL2	UniProtKB: P19875	5209	5210	5211, 5212, 5213, 5214, 5215, 5216
CXCL3	UniProtKB: P19876	5217	5218	5219, 5220, 5221, 5222, 5223, 5224
CXCL4	UniProtKB: P02776	5225	5226	5227, 5228, 5229, 5230, 5231, 5232
CXCL5	UniProtKB: P42830	5233	5234	5235, 5236, 5237, 5238, 5239, 5240

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	RNA Sequence SEQ ID NO:
CXCL5	UniProtKB: Q6I9S7	5241	5242	5243, 5244, 5245, 5246, 5247, 5248
CXCL6	UniProtKB: P80162	5249	5250	5251, 5252, 5253, 5254, 5255, 5256
CXCL7	UniProtKB: P02775	5257	5258	5259, 5260, 5261, 5262, 5263, 5264
CXCL8	UniProtKB: P10145	5265	5266	5267, 5268, 5269, 5270, 5271, 5272
CXCL9	UniProtKB: L8E8X0	5273	5274	5275, 5276, 5277, 5278, 5279, 5280
CXCL9	UniProtKB: Q07325	5281	5282	5283, 5284, 5285, 5286, 5287, 5288
XCL1	UniProtKB: P47992	5289	5290	5291, 5292, 5293, 5294, 5295, 5296
XCL2	UniProtKB: Q9UBD3	5297	5298	5299, 5300, 5301, 5302, 5303, 5304

In this context particularly preferred are chemokines chosen from the following list: CXCL9, CXCL10, CCL5, XCL1, CCL20, CCL19, CCL21, CCL2, CCL3, CCL16, and CXCL12.

According to the present invention, in a more preferred embodiment, the inventive composition comprises at least one RNA, preferably an mRNA comprising at least one coding region encoding at least one chemokine or a fragment or variant thereof, wherein the at least one coding region comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequences according to the SEQ ID Nos as disclosed in Table 2.

3. Suicide gene products

In a further preferred embodiment of the inventive RNA containing composition the RNA codes for at least one so-called suicide gene product, especially for a suicide enzyme, preferably for a nucleotide metabolizing enzyme. Preferably the RNA is used in combination with a prodrug which is a substrate of the suicide gene product, especially the suicide enzyme, and which is converted to a cytotoxic compound by the suicide gene product. The appropriate prodrug may be added to the inventive RNA composition or it may be administered separately to the patient.

One or more preferred suicide enzymes may be chosen from the following list: thymidine kinase, preferably a viral thymidine kinase, more preferrably *Herpes simplex* virus thymidine kinase, *Varicella zoster* thymidine kinase; a plant thymidine kinase, preferably a tomato thymidine kinase; cytosine deaminase, preferably bacterial cytosine deaminase or Yeast cytosine deaminase; deoxynucleoside kinase, preferably *Drosophila melanogaster* deoxynucleoside kinase; deoxycytidine kinase, preferably a mammalian deoxycytidine kinase, purine nucleoside phosphorylase, preferably a bacterial purine nucleoside phosphorylase.

It is already known that suicide gene therapy is a promising treatment for cancer (Ardiani et al., 2012. Curr. Gene Ther. 12(2):77-91. PMID: 22384805). This approach is based on the successful delivery and expression of the suicide gene in tumor cells. The suicide gene encodes an enzyme with the unique ability to activate an otherwise ineffective prodrug. Following suicide gene expression in transfected cells, an appropriate prodrug is administered and is converted to a cytotoxic compound by the actions of the suicide gene product. As most suicide genes encode enzymes belonging to the class of nucleotide metabolizing enzymes, the general mode of action of activated prodrugs is interference with DNA synthesis that consequently results in induction of apoptosis. The potency of these drugs is maximized in cancer cells due to their greater proliferative rate as compared to normal cells. Because of the prospect to preferentially deliver genes to tumor cells, this strategy has the potential to offer selective tumor killing while sparing normal cells, a feature that standard chemotherapeutic and radiotherapy approaches do not generally afford.

The following table 3 (Ardiani et al., 2012. Curr. Gene Ther. 12(2):77-91. PMID: 22384805) summarizes preferred nucleotide metabolizing enzymes usable in the inventive approach. The table includes variants and mutants of such enzymes which were generated by protein engineering strategies.

Table 3: Suicide enzymes

Enzyme	Source gene	Natural substrate	Prodrug	Variants/ Mutants	Drug inhibi-tors action*
Herpes Simplex	Herpes Simplex Virus 1	Thymidine	Ganciclovir (GCV), acyclovir (ACV)	Mutant 30 Mutant 75	1 1

Enzyme	Source gene	Natural substrate	Prodrug	Variants/ Mutants	Drug inhibi-tors action*
Virus Thymidine Kinase (HSVTK)	(HSV-1) Thymidine Kinase (TK)			SR39 A168H A167Y Q125N	1 1 1 1, 2
Bacterial Cytosine Deaminase (bCD)	*Escherichia coli* –codA	Cytosine	5-Fluorocytosine (5-FC)	D314 mutants bCD1525	1, 2, 4 1, 2, 4
Yeast Cytosine Deaminase (yCD)	*Saccharo-myces cerevisiae* –fcy1	Cytosine	5-FC	yCD triple D92E	1, 2, 4 1, 2, 4
Drosophila melano-gaster Deoxynucleoside Kinase (Dm-dNK)	*Drosophila melanogaster* –dNK	All four deoxy-ribo-nucleo-sides	azidothymidine (AZT), dideoxycytoinse (ddC); CdA; 9-beta-D-arabinofuranosyl-2-fluoroadenine (F-AraA); GCV, 9-beta-D-arabinosylguanine (AraG); 2',3'-didehydro-3'-deoxythymidine (D4T); 2',3'-Dideoxythymidine (ddT)	MuD B5 B10 M88R HDHD-12, HD-16 R4.V3	1, 5 1 1, 3 1 1, 5 1
Deoxy-cytidine Kinase (dCK)	*Homo sapiens* –dCK	Deoxy-cytidine	2',2'-difluoro-deoxycytidine (dFdC), AraA, β-L-thymidine (L-dT) AZT cytarabine 5'-monophosphate (AraC)	DMMA, DMLA EpTK6 Ser-74	1, 3 1, 3, 5 1, 3
Purine Nucleoside Phospho-rylase (PNP)	*Escherichia coli* -deoD	Purine ribo-nucleo-sides	9-(6-deoxy-α-L-talofuranosyl)-6-methylpurine (Me(talo)-MeP-R)	M64V	1, 4

* Drug inhibitory action. 1: DNA synthesis; 2: Thymidylate synthetase; 3: Ribonucleotide reductase; 4: RNA/protein synthesis; 5: Reverse transcriptase.

Herpes simplex virus type 1 thymidine kinase (HSVTK, EC 2.7.1.21), a homodimer with a subunit molecular mass of 45 kDa, is responsible for the phosphorylation of thymidine, deoxycytidine, deoxythymidylate (dTMP) as well as various pharmaceutically important pyrimidine and guanosine analogs. Of particular note, HSVTK is responsible for the initial and rate limiting phosphorylation of the antiviral guanosine analogs acyclovir (ACV) and ganciclovir (GCV). Once monophosphorylated these analogs can be further phosphorylated by endogenous enzymes (guanylate kinase and nucleoside diphosphokinase) before being incorporated into nascent DNA to cause double strand destabilization and, subsequently, cell death.

Moreover, the *Varicella zoster* virus thymidine kinase (VZV-tk) may be used e.g. in conjunction with the prodrug 6-methoxypurine arabinoside (ara-M) or 1-(2'-deoxy-2-flioro-b-D-arabinofuranosyl)-5-iodouracil (FIAU). Other examples are thymidine kinases of Aleutian disease virus (ADV), respiratory syncytial virus (RSV) and cytomegalovirus (CMV).

Cytosine deaminase (CD; EC 3.5.4.1) is an enzyme in the pyrimidine salvage pathway that catalyzes the deamination of cytosine to form uracil and ammonia. CD from *E. coli* (bCD) is a hexamer of 48 kDA subunits with a catalytic metal iron. This enzyme is absent in mammals and uniquely present in fungi and bacteria. It is used in suicide gene therapy because of its ability to deaminate the anti-fungal drug, 5-fluorocytosine (5FC), to 5-fluorouracil (5FU), a potent anti-neoplastic drug. UPRT (Uracil phosphoribosyltransferase) may be used as potential enhancer.

Saccharomyces cerevisiae or Yeast cytosine deaminase (yCD, EC 3.5.4.1) is a homodimer of 17.5 kDa subunits and has been shown to be more active towards 5FC than bCD (22-fold lower K_m) with a slightly better catalytic efficiency (k_{cat}/K_m) toward 5FC relative to its natural substrate cytosine.

Drosophila melanogaster deoxyribonucleoside kinase (Dm-dNK; EC 2.7.1.145) is a 29 kDa homodimeric, multisubstrate kinase able to phosphorylate all four natural deoxyribonucleosides required for DNA synthesis. In addition to its broad substrate specificity, Dm-dNK exhibits higher catalytic rates toward these natural deoxynucleosides and several nucleoside analogs as compared

to mammalian deoxynucleoside kinases. Due to these distinctive characteristics Dm-dNK is a especially preferred enzyme for the inventive suicide gene therapy application.

Human deoxycytidine kinase (dCK; EC 2.7.1.74) is a 30.5 kDa homodimeric enzyme in the salvage pathway of deoxyribonucleosides and is responsible for activating all natural deoxyribonucleosides, excluding thymidine, as precursors for DNA synthesis. Due to its broad substrate specificity, dCK is able to activate multiple nucleoside analogs effective against different types of cancer. However, wild type dCK is intrinsically a relatively poor catalyst with low turnover rates and prodrug activation is dependent on its expression levels. Indeed, nucleoside analogs that are efficient substrates of dCK, such as cytarabine (AraC), fludarabine (F-AraA), cladribine (CdA), and gemcitabine (dFdC), are effective anti-leukemic agents as lymphoblasts have been shown to have high dCK expression levels whereas cancer cells lacking dCK activity are resistant to these same analogs. Therefore dCK is an especially preferred enzyme for the inventive approach.

Preferably the RNA of the inventive RNA containing composition is used in combination with further components which enhance the cytotoxic effect of the treatment. It is especially preferred to use the RNA in combination with RNA coding for connexins and/or with a protein of the connexin family or parts or fragments thereof. Connexins are transmembrane proteins which form gap junctions between cells. They allow transfer of e.g. molecules between neighboring cells thereby enabling the transfer of cytoxic substances.

Although suicide gene therapy is a fairly new anti-cancer approach, the concept was originally described more than two decades ago in 1986 by Moolten (Moolten, 1986. Cancer Res. 46(10):5276-81). He also proposed the existence of what is currently known as the bystander effect, now widely recognized as a fundamental feature of suicide gene therapy success. By definition the bystander effect is the extension of cytotoxic effects from transfected cells to non-transfected neighboring cells such that complete tumor regression is observed when only a small subpopulation of tumor cells is successfully transfected. This phenomenon is crucial to the overall effectiveness of suicide gene therapy today due to low transfection efficiencies achievable by available delivery systems.

The bystander effect is thought to occur via two major mechanisms: local and immune-mediated. The local mechanism involves the killing of untransfected nearby cells due to the transfer of toxic materials or suicide enzymes through gap junctions, apoptotic vesicles or through diffusion of soluble toxic metabolites. Gap junctions are important in cell-cell interactions and are responsible for the transfer of ions, nucleotides and small molecules to adjacent cells. The transfer of toxic drugs through gap junctions, however, may not be available in certain types of tumors that down regulate intracellular gap junction communication and display disorganized and non-functional gap junctions. To address this problem, several studies have increased the expression of connexins, the building blocks of gap junctions, and demonstrated that enhanced bystander and cell killing effects were achieved.

Accumulating evidence from *in vivo* experiments suggests that the immune-mediated bystander effect plays an important role in tumor regression as well. The presence of inflammatory infiltrates, chemokines, and cytokines have been found elevated in regressing tumors of immune competent animals receiving suicide gene therapy treatment. These cytokines and chemokines further induce the production of immune-regulatory molecules able to stimulate a more robust anti-cancer effect and additionally, because death of transfected cells is through apoptosis, numerous inflammatory signals may be released to evoke a potent immune response. Therefore the combination of the inventive composition with connexins or with RNA coding for connexins is especially preferred, because it strengthens the bystander effect thereby increasing the efficiency of the inventive RNA containing composition.

According to preferred embodiments in the context of the present invention suicide gene products may be selected from any suicide gene product selected from the group consisting of Cytosine_Deaminase_codA; Cytosine_Deaminase_fcy1; Deoxy-cytidine_Kinase_dCK; Deoxynucleoside_Kinase_dNK; Purine_Nucleoside_Phosphorylase_deoD; Thymidine_Kinase_TK, preferably as disclosed in Table 4. Particularly preferred in this context are the RNA sequences encoding a suicide gene product according to Table 4.

Table 4: Suicide Gene Products

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	RNA Sequence SEQ ID NO:
Cytosine_Deaminase_codA	UniProtKB: A0A024KS17	5305	5306	5307, 5308, 5309, 5310, 5311, 5312
Cytosine_Deaminase_codA	UniProtKB: A0A0H2V4N7	5313	5314	5315, 5316, 5317, 5318, 5319, 5320
Cytosine_Deaminase_codA	UniProtKB: A0A0H2YX33	5321	5322	5323, 5324, 5325, 5326, 5327, 5328
Cytosine_Deaminase_codA	UniProtKB: F4NM90	5329	5330	5331, 5332, 5333, 5334, 5335, 5336
Cytosine_Deaminase_codA	UniProtKB: H9UNZ4	5337	5338	5339, 5340, 5341, 5342, 5343, 5344
Cytosine_Deaminase_codA	UniProtKB: Q1RFJ5	5345	5346	5347, 5348, 5349, 5350, 5351, 5352
Cytosine_Deaminase_codA	UniProtKB: Q2VP09	5353	5354	5355, 5356, 5357, 5358, 5359, 5360
Cytosine_Deaminase_codA	UniProtKB: Q53ZC8	5361	5362	5363, 5364, 5365, 5366, 5367, 5368
Cytosine_Deaminase_codA	UniProtKB: Q6Q8Q1	5369	5370	5371, 5372, 5373, 5374, 5375, 5376
Cytosine_Deaminase_codA	UniProtKB: W8ZNH5	5377	5378	5379, 5380, 5381, 5382, 5383, 5384
Cytosine_Deaminase_fcy1	UniProtKB: A0A023ZJG6	5385	5386	5387, 5388, 5389, 5390, 5391, 5392
Cytosine_Deaminase_fcy1	UniProtKB: A0A024XGF7	5393	5394	5395, 5396, 5397, 5398, 5399, 5400
Cytosine_Deaminase_fcy1	UniProtKB: A0A024XUW9	5401	5402	5403, 5404, 5405, 5406, 5407, 5408
Cytosine_Deaminase_fcy1	UniProtKB: A0A0C5ITD0	5409	5410	5411, 5412, 5413, 5414, 5415, 5416
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4WVI5	5417	5418	5419, 5420, 5421, 5422, 5423, 5424
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4WY08	5425	5426	5427, 5428, 5429, 5430, 5431, 5432
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4WZA2	5433	5434	5435, 5436, 5437, 5438, 5439, 5440
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4WZQ5	5441	5442	5443, 5444, 5445, 5446, 5447, 5448
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4X0R8	5449	5450	5451, 5452, 5453, 5454, 5455, 5456
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4X195	5457	5458	5459, 5460, 5461, 5462, 5463, 5464

Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4X2R9	5465	5466	5467, 5468, 5469, 5470, 5471, 5472
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4X3Q1	5473	5474	5475, 5476, 5477, 5478, 5479, 5480
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4X4K1	5481	5482	5483, 5484, 5485, 5486, 5487, 5488
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4X5B7	5489	5490	5491, 5492, 5493, 5494, 5495, 5496
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4X7R4	5497	5498	5499, 5500, 5501, 5502, 5503, 5504
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4X7X4	5505	5506	5507, 5508, 5509, 5510, 5511, 5512
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XA07	5513	5514	5515, 5516, 5517, 5518, 5519, 5520
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XA25	5521	5522	5523, 5524, 5525, 5526, 5527, 5528
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XAV6	5529	5530	5531, 5532, 5533, 5534, 5535, 5536
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XCJ5	5537	5538	5539, 5540, 5541, 5542, 5543, 5544
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XDL4	5545	5546	5547, 5548, 5549, 5550, 5551, 5552
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XG53	5553	5554	5555, 5556, 5557, 5558, 5559, 5560
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XGH3	5561	5562	5563, 5564, 5565, 5566, 5567, 5568
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XHD4	5569	5570	5571, 5572, 5573, 5574, 5575, 5576
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XIK5	5577	5578	5579, 5580, 5581, 5582, 5583, 5584
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XJR4	5585	5586	5587, 5588, 5589, 5590, 5591, 5592
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XL36	5593	5594	5595, 5596, 5597, 5598, 5599, 5600
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XNH2	5601	5602	5603, 5604, 5605, 5606, 5607, 5608
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XNS1	5609	5610	5611, 5612, 5613, 5614, 5615, 5616
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XQY5	5617	5618	5619, 5620, 5621, 5622, 5623, 5624
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XS80	5625	5626	5627, 5628, 5629, 5630, 5631, 5632
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XS82	5633	5634	5635, 5636, 5637, 5638, 5639, 5640
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XTC2	5641	5642	5643, 5644, 5645, 5646, 5647, 5648

Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XUZ4	5649	5650	5651, 5652, 5653, 5654, 5655, 5656
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XW26	5657	5658	5659, 5660, 5661, 5662, 5663, 5664
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XXD1	5665	5666	5667, 5668, 5669, 5670, 5671, 5672
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XYH3	5673	5674	5675, 5676, 5677, 5678, 5679, 5680
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4XZT0	5681	5682	5683, 5684, 5685, 5686, 5687, 5688
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Y164	5689	5690	5691, 5692, 5693, 5694, 5695, 5696
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Y2A8	5697	5698	5699, 5700, 5701, 5702, 5703, 5704
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Y3N1	5705	5706	5707, 5708, 5709, 5710, 5711, 5712
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Y5S3	5713	5714	5715, 5716, 5717, 5718, 5719, 5720
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Y5Y1	5721	5722	5723, 5724, 5725, 5726, 5727, 5728
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Y7I2	5729	5730	5731, 5732, 5733, 5734, 5735, 5736
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Y8S5	5737	5738	5739, 5740, 5741, 5742, 5743, 5744
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YAR2	5745	5746	5747, 5748, 5749, 5750, 5751, 5752
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YBY2	5753	5754	5755, 5756, 5757, 5758, 5759, 5760
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YCB3	5761	5762	5763, 5764, 5765, 5766, 5767, 5768
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YEC2	5769	5770	5771, 5772, 5773, 5774, 5775, 5776
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YF30	5777	5778	5779, 5780, 5781, 5782, 5783, 5784
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YGU2	5785	5786	5787, 5788, 5789, 5790, 5791, 5792
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YHN3	5793	5794	5795, 5796, 5797, 5798, 5799, 5800
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YIU4	5801	5802	5803, 5804, 5805, 5806, 5807, 5808
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YJ74	5809	5810	5811, 5812, 5813, 5814, 5815, 5816
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YKC5	5817	5818	5819, 5820, 5821, 5822, 5823, 5824
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YMN8	5825	5826	5827, 5828, 5829, 5830, 5831, 5832

Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YMV6	5833	5834	5835, 5836, 5837, 5838, 5839, 5840
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YPP6	5841	5842	5843, 5844, 5845, 5846, 5847, 5848
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YRD4	5849	5850	5851, 5852, 5853, 5854, 5855, 5856
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YS13	5857	5858	5859, 5860, 5861, 5862, 5863, 5864
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YTJ7	5865	5866	5867, 5868, 5869, 5870, 5871, 5872
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YUX9	5873	5874	5875, 5876, 5877, 5878, 5879, 5880
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YV34	5881	5882	5883, 5884, 5885, 5886, 5887, 5888
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YXE1	5889	5890	5891, 5892, 5893, 5894, 5895, 5896
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YYM6	5897	5898	5899, 5900, 5901, 5902, 5903, 5904
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4YZB7	5905	5906	5907, 5908, 5909, 5910, 5911, 5912
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Z060	5913	5914	5915, 5916, 5917, 5918, 5919, 5920
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Z1S2	5921	5922	5923, 5924, 5925, 5926, 5927, 5928
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Z2L6	5929	5930	5931, 5932, 5933, 5934, 5935, 5936
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Z4A1	5937	5938	5939, 5940, 5941, 5942, 5943, 5944
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Z552	5945	5946	5947, 5948, 5949, 5950, 5951, 5952
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Z6N6	5953	5954	5955, 5956, 5957, 5958, 5959, 5960
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Z800	5961	5962	5963, 5964, 5965, 5966, 5967, 5968
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4Z9V2	5969	5970	5971, 5972, 5973, 5974, 5975, 5976
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZB52	5977	5978	5979, 5980, 5981, 5982, 5983, 5984
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZCA2	5985	5986	5987, 5988, 5989, 5990, 5991, 5992
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZCG3	5993	5994	5995, 5996, 5997, 5998, 5999, 6000
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZEM2	6001	6002	6003, 6004, 6005, 6006, 6007, 6008
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZFD0	6009	6010	6011, 6012, 6013, 6014, 6015, 6016

Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZGR1	6017	6018	6019, 6020, 6021, 6022, 6023, 6024
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZIM2	6025	6026	6027, 6028, 6029, 6030, 6031, 6032
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZJC0	6033	6034	6035, 6036, 6037, 6038, 6039, 6040
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZK17	6041	6042	6043, 6044, 6045, 6046, 6047, 6048
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZMC8	6049	6050	6051, 6052, 6053, 6054, 6055, 6056
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZMX9	6057	6058	6059, 6060, 6061, 6062, 6063, 6064
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZP21	6065	6066	6067, 6068, 6069, 6070, 6071, 6072
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZQ62	6073	6074	6075, 6076, 6077, 6078, 6079, 6080
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZQ92	6081	6082	6083, 6084, 6085, 6086, 6087, 6088
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZS31	6089	6090	6091, 6092, 6093, 6094, 6095, 6096
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZS87	6097	6098	6099, 6100, 6101, 6102, 6103, 6104
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZTS6	6105	6106	6107, 6108, 6109, 6110, 6111, 6112
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZUK0	6113	6114	6115, 6116, 6117, 6118, 6119, 6120
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZVN6	6121	6122	6123, 6124, 6125, 6126, 6127, 6128
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZWP2	6129	6130	6131, 6132, 6133, 6134, 6135, 6136
Cytosine_Deaminase_fcy1	UniProtKB: A0A0D4ZX07	6137	6138	6139, 6140, 6141, 6142, 6143, 6144
Cytosine_Deaminase_fcy1	UniProtKB: Q12178	6145	6146	6147, 6148, 6149, 6150, 6151, 6152
Cytosine_Deaminase_fcy1	UniProtKB: W7PK48	6153	6154	6155, 6156, 6157, 6158, 6159, 6160
Cytosine_Deaminase_fcy1	UniProtKB: W7R647	6161	6162	6163, 6164, 6165, 6166, 6167, 6168
Deoxy-cytidine_Kinase_dCK	UniProtKB: P27707	6169	6170	6171, 6172, 6173, 6174, 6175, 6176
Deoxynucleoside_Kinase_dNK	UniProtKB: Q540Z9	6177	6178	6179, 6180, 6181, 6182, 6183, 6184
Deoxynucleoside_Kinase_dNK	UniProtKB: Q9XZT6	6185	6186	6187, 6188, 6189, 6190, 6191, 6192
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A023Z7B9	6193	6194	6195, 6196, 6197, 6198, 6199, 6200

Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A024KMI2	6201	6202	6203, 6204, 6205, 6206, 6207, 6208
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0E0SRY5	6209	6210	6211, 6212, 6213, 6214, 6215, 6216
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0E0U7I4	6217	6218	6219, 6220, 6221, 6222, 6223, 6224
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0E0VFI3	6225	6226	6227, 6228, 6229, 6230, 6231, 6232
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0E0Y455	6233	6234	6235, 6236, 6237, 6238, 6239, 6240
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0E1M7E2	6241	6242	6243, 6244, 6245, 6246, 6247, 6248
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0E3KJD7	6249	6250	6251, 6252, 6253, 6254, 6255, 6256
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0F6CCW6	6257	6258	6259, 6260, 6261, 6262, 6263, 6264
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0F6FGI8	6265	6266	6267, 6268, 6269, 6270, 6271, 6272
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0F6GWR2	6273	6274	6275, 6276, 6277, 6278, 6279, 6280
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0G2SIK5	6281	6282	6283, 6284, 6285, 6286, 6287, 6288
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0G3J9R6	6289	6290	6291, 6292, 6293, 6294, 6295, 6296
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0G3J9Y2	6297	6298	6299, 6300, 6301, 6302, 6303, 6304
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0G3KD68	6305	6306	6307, 6308, 6309, 6310, 6311, 6312
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0H2Z6H1	6313	6314	6315, 6316, 6317, 6318, 6319, 6320
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0H3EQW1	6321	6322	6323, 6324, 6325, 6326, 6327, 6328
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0H3XF09	6329	6330	6331, 6332, 6333, 6334, 6335, 6336
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A0A0J9WZC9	6337	6338	6339, 6340, 6341, 6342, 6343, 6344
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A7ZVS7	6345	6346	6347, 6348, 6349, 6350, 6351, 6352
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: A8A8B3	6353	6354	6355, 6356, 6357, 6358, 6359, 6360
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B1IS35	6361	6362	6363, 6364, 6365, 6366, 6367, 6368
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B1LEI9	6369	6370	6371, 6372, 6373, 6374, 6375, 6376
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B1XFJ4	6377	6378	6379, 6380, 6381, 6382, 6383, 6384

Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B3HEI4	6385	6386	6387, 6388, 6389, 6390, 6391, 6392
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B5Z4R6	6393	6394	6395, 6396, 6397, 6398, 6399, 6400
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B6I6N1	6401	6402	6403, 6404, 6405, 6406, 6407, 6408
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B7LEN0	6409	6410	6411, 6412, 6413, 6414, 6415, 6416
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B7LXU6	6417	6418	6419, 6420, 6421, 6422, 6423, 6424
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B7MNJ1	6425	6426	6427, 6428, 6429, 6430, 6431, 6432
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B7N2V8	6433	6434	6435, 6436, 6437, 6438, 6439, 6440
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B7NH52	6441	6442	6443, 6444, 6445, 6446, 6447, 6448
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B7NW64	6449	6450	6451, 6452, 6453, 6454, 6455, 6456
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: B7UR12	6457	6458	6459, 6460, 6461, 6462, 6463, 6464
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: C3SE47	6465	6466	6467, 6468, 6469, 6470, 6471, 6472
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: C4ZT66	6473	6474	6475, 6476, 6477, 6478, 6479, 6480
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: C8TQD7	6481	6482	6483, 6484, 6485, 6486, 6487, 6488
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: C8U157	6489	6490	6491, 6492, 6493, 6494, 6495, 6496
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: C8UN92	6497	6498	6499, 6500, 6501, 6502, 6503, 6504
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: D3GY24	6505	6506	6507, 6508, 6509, 6510, 6511, 6512
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: D3QNE6	6513	6514	6515, 6516, 6517, 6518, 6519, 6520
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: D6I4N2	6521	6522	6523, 6524, 6525, 6526, 6527, 6528
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: D6IHU2	6529	6530	6531, 6532, 6533, 6534, 6535, 6536
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: D6J6A4	6537	6538	6539, 6540, 6541, 6542, 6543, 6544
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: E0J437	6545	6546	6547, 6548, 6549, 6550, 6551, 6552
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: E2QLE4	6553	6554	6555, 6556, 6557, 6558, 6559, 6560
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: E3PFG7	6561	6562	6563, 6564, 6565, 6566, 6567, 6568

Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: E8YEH0	6569	6570	6571, 6572, 6573, 6574, 6575, 6576
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: F4NLK2	6577	6578	6579, 6580, 6581, 6582, 6583, 6584
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: F4SEX7	6585	6586	6587, 6588, 6589, 6590, 6591, 6592
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: F4STB8	6593	6594	6595, 6596, 6597, 6598, 6599, 6600
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: F4T9F1	6601	6602	6603, 6604, 6605, 6606, 6607, 6608
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: F4UXB7	6609	6610	6611, 6612, 6613, 6614, 6615, 6616
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: F4VN60	6617	6618	6619, 6620, 6621, 6622, 6623, 6624
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: F4VQF8	6625	6626	6627, 6628, 6629, 6630, 6631, 6632
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: H9V0H4	6633	6634	6635, 6636, 6637, 6638, 6639, 6640
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: J7QV83	6641	6642	6643, 6644, 6645, 6646, 6647, 6648
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: P0ABP8	6649	6650	6651, 6652, 6653, 6654, 6655, 6656
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: Q0T8S9	6657	6658	6659, 6660, 6661, 6662, 6663, 6664
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: Q1R259	6665	6666	6667, 6668, 6669, 6670, 6671, 6672
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: W8ZSE4	6673	6674	6675, 6676, 6677, 6678, 6679, 6680
Purine_Nucleoside_ Phosphorylase_deoD	UniProtKB: X5FDR9	6681	6682	6683, 6684, 6685, 6686, 6687, 6688
Thymidine_Kinase_TK	UniProtKB: B2CPP5	6689	6690	6691, 6692, 6693, 6694, 6695, 6696
Thymidine_Kinase_TK	UniProtKB: B2CPP6	6697	6698	6699, 6700, 6701, 6702, 6703, 6704
Thymidine_Kinase_TK	UniProtKB: B2CPP7	6705	6706	6707, 6708, 6709, 6710, 6711, 6712
Thymidine_Kinase_TK	UniProtKB: B2CPP8	6713	6714	6715, 6716, 6717, 6718, 6719, 6720
Thymidine_Kinase_TK	UniProtKB: B2CPP9	6721	6722	6723, 6724, 6725, 6726, 6727, 6728
Thymidine_Kinase_TK	UniProtKB: B2CPQ0	6729	6730	6731, 6732, 6733, 6734, 6735, 6736
Thymidine_Kinase_TK	UniProtKB: B2CPQ2	6737	6738	6739, 6740, 6741, 6742, 6743, 6744
Thymidine_Kinase_TK	UniProtKB: B2CPQ3	6745	6746	6747, 6748, 6749, 6750, 6751, 6752

Thymidine_Kinase_TK	UniProtKB: B2CPQ4	6753	6754	6755, 6756, 6757, 6758, 6759, 6760
Thymidine_Kinase_TK	UniProtKB: B2CPQ5	6761	6762	6763, 6764, 6765, 6766, 6767, 6768
Thymidine_Kinase_TK	UniProtKB: O72346	6769	6770	6771, 6772, 6773, 6774, 6775, 6776
Thymidine_Kinase_TK	UniProtKB: P06478	6777	6778	6779, 6780, 6781, 6782, 6783, 6784
Thymidine_Kinase_TK	UniProtKB: P08333	6785	6786	6787, 6788, 6789, 6790, 6791, 6792
Thymidine_Kinase_TK	UniProtKB: Q9DLP2	6793	6794	6795, 6796, 6797, 6798, 6799, 6800
Thymidine_Kinase_TK	UniProtKB: Q9ENS0	6801	6802	6803, 6804, 6805, 6806, 6807, 6808
Thymidine_Kinase_TK	UniProtKB: Q9ENS1	6809	6810	6811, 6812, 6813, 6814, 6815, 6816
Thymidine_Kinase_TK	UniProtKB: Q9ENS2	6817	6818	6819, 6820, 6821, 6822, 6823, 6824
Thymidine_Kinase_TK	UniProtKB: Q9ENS3	6825	6826	6827, 6828, 6829, 6830, 6831, 6832
Thymidine_Kinase_TK	UniProtKB: Q9ENS4	6833	6834	6835, 6836, 6837, 6838, 6839, 6840
Thymidine_Kinase_TK	UniProtKB: Q9ENS5	6841	6842	6843, 6844, 6845, 6846, 6847, 6848
Thymidine_Kinase_TK	UniProtKB: Q9IYZ7	6849	6850	6851, 6852, 6853, 6854, 6855, 6856
Thymidine_Kinase_TK	UniProtKB: Q9IYZ9	6857	6858	6859, 6860, 6861, 6862, 6863, 6864
Thymidine_Kinase_TK	UniProtKB: Q9IZ02	6865	6866	6867, 6868, 6869, 6870, 6871, 6872
Thymidine_Kinase_TK	UniProtKB: Q9IZ03	6873	6874	6875, 6876, 6877, 6878, 6879, 6880
Thymidine_Kinase_TK	UniProtKB: Q9IZ07	6881	6882	6883, 6884, 6885, 6886, 6887, 6888
Thymidine_Kinase_TK	UniProtKB: Q9QNF7	6889	6890	6891, 6892, 6893, 6894, 6895, 6896

According to the present invention, in a more preferred embodiment, the inventive composition comprises at least one RNA, preferably an mRNA comprising at least one coding region encoding at least one suicide gene product or a fragment or variant thereof, wherein the at least one coding region comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequences according to the SEQ ID Nos as disclosed in Table 4.

4. Immunogenic proteins or peptides

Preferably the RNA, preferably mRNA of the inventive RNA composition codes for at least one immunogenic protein or peptide, especially a protein or peptide of a pathogen, preferably a viral pathogen, or a fragment or variant thereof. By using RNA which codes for an immunogenic protein or peptide which is preferably a pathogenic antigen it is possible to utilize preexisting immunity against such antigens for treatment of tumor and/or cancer diseases. The memory immune response is triggered and the immune system is strengthened for attacking tumor cells.

This embodiment of the invention is based on the recognition that in principle every organism with an immune system exhibits "memory immune responses" against certain foreign molecules (antigens), for example proteins, in particular viral or bacterial proteins. If an organism has already been infected at an earlier point in time with the antigen an immune response against e.g. the viral protein has already been triggered by this infection. The immune system has a "memory" of this response and stores it. As consequence of a reinfection with the antigen the immune response is reactivated. Such reactivation may proceed by administration of an RNA, preferably mRNA coding for the antigen, wherein the preferred intratumoral administration according to the invention is especially effective. By reactivation of the memory immune response against e.g. viral pathogens it is possible to destroy tumor cells effectively.

Preferred examples of immunogenic proteins or peptides for this embodiment of the invention are proteins or peptides of widespread pathogens, i.e. pathogens with which every organism, in particular mammals, preferably humans, has a high probability of being infected at least once in his/her lifetime. These include, for example, any structural or non-structural protein or peptide of:

- influenza virus type A or B or any other orthomyxovirus (influenza type C),
- picornaviruses, such as rhinovirus or hepatitis A virus,
- togaviruses, such as alphavirus or rubivirus, e.g. Sindbis, Semliki-Forest or rubeolavirus (measles virus),
- rubella virus (German measles virus),
- coronaviruses, in particular subtypes HCV-229E or HCV-OC43,
- rhabdoviruses, such as rabies virus,

- paramyxoviruses, such as mumps virus,
- reoviruses, such as group A, B or C rotavirus,
- hepadnaviruses, such as hepatitis B virus,
- papoviruses, such as human papillomaviruses (HPV) of any serotype, especially from 1 to 75,
- adenoviruses, in particular type 1 to 47,
- herpesviruses, such as Herpes simplex virus 1, 2 or 3,
- cytomegalovirus (CMV), preferably CMVpp65,
- Epstein Barr virus (EBV),
- vaccinia viruses and
- the bacterium *Chlamydophila pneumoniae* (*Chlamydia pneumoniae*).

Further examples of preferred immunogenic proteins or peptides are proteins or peptides of pathogens which only seldom infect an organism. Nevertheless RNA coding for one or more of these proteins or peptides may be effective in the inventive approach. These proteins or peptide include, for example, any structural or non-structural protein or peptide of:

- Flaviviruses, such as dengue virus type 1 to 4, yellow fever virus, West Nile virus, Japanese encephalitis virus
- hepatitis C virus,
- caliciviruses,
- filoviruses, such as Ebola virus,
- bornaviruses,
- bunyaviruses, such as Rift Valley fever virus,
- arenaviruses, such as LCMV (lymphocytic choriomeningitis virus) or hemorrhagic fever viruses,
- retroviruses, such as HIV and
- parvoviruses.

Preferably the RNA of the inventive mRNA composition codes for influenza nucleoprotein (NP). It has been shown by the inventors that the use of a composition containing mRNA coding for influenza nucleoprotein is especially effective in reducing tumor size, when applied according to the

inventive approach. In this context an mRNA encoding an Influenza nucleoprotein according to SEQ ID NO. 6 is particularly preferred.

5. Cell death inducers and apoptosis inducers:

In the broadest sense, an apoptosis inducer or cell death inducer has to be understood as a molecule inducing autophagy, cornification, excitotoxicity, necrosis, Wallerian degeneration, entosis, mitotic catastrophe, necroptosis and pyroptosis (reviewed in Kroemer, G., et al. "Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009." Cell Death & Differentiation 16.1 (2009): 3-11.).

In a further preferred embodiment of the inventive RNA containing composition the RNA codes for at least one apoptosis inducer, preferably an apoptosis inducer chosen from the group consisting of the Bcl-2 family and tumor suppressor protein p53 and ligands of transmembrane death receptors, especially the TNF (tumor necrosis factor) receptor gene superfamily, pro-apoptic receptor agonists and Beclin-1.

A particularily preferred apoptosis inducer in the context of the present invention is Beclin-1 (derived from the BECN1 gene). It is known in the art that Beclin-1 interacts with Bcl-2, BCL2L2, GOPC and MAP1LC3A to regulate autophagy and cell death.

Apoptosis provides an important barrier against cancer. However, specific mutations (e.g. mutation of the tumor suppressor gene p53) enable some tumor cells to escape apoptotic death and become more malignant. By using an mRNA coding for at least one apoptosis inducer it is possible to reactivate apoptosis which is an important and effective system of the organism to eliminate cancer cells.

Preferred examples of apoptosis inducers may be chosen from the following list: Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, Blk, Cytochrome c, Caspases, especially Caspase 3, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Death domain, especially of Fas, preferably FasL, TNFα, Apo2L/TRAIL, agonist of DR4 and/or DR5, Apo3L, DR4 agonistic antibody, DR5 agonistic antibody, protein kinase R (PKR) (preferably constitutive active PKR), Granzyme B.

Two signalling pathways initiate apoptosis: the intrinsic pathway acts through intracellular Bcl-2 proteins, the extrinsic pathway through cell-surface pro-apoptotic receptors.

The intrinsic signaling pathway for programmed cell death involves non-receptor–mediated intracellular signals, inducing activities in the mitochondria that initiate apoptosis. Stimuli for the intrinsic pathway include viral infections or damage to the cell by toxins, free radicals, or radiation. Damage to the cellular DNA can also induce the activation of the intrinsic pathway for programmed cell death. These stimuli induce changes in the inner mitochondrial membrane that result in the loss of transmembrane potential, causing the release of pro-apoptotic proteins into the cytosol. Pro-apoptotic proteins activate caspases that mediate the destruction of the cell through many pathways. These proteins also translocate into the cellular nucleus, inducing DNA fragmentation, a hallmark of apoptosis. The regulation of pro-apoptotic events in the mitochondria occurs through activity of members of the Bcl-2 family of proteins and the tumor suppressor protein p53. Members of the Bcl-2 family of proteins may be pro-apoptotic or anti-apoptotic. The anti-apoptotic proteins are Bcl-2, Bcl-x, Bcl-xL, Bcl-XS, Bcl-w, and BAG. Pro-apoptotic proteins include Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk (Elmore, 2007. Toxicol Pathol. 35(4):495-516 (PMID: 17562483)), which are especially preferred for the inventive approach.

The extrinsic signaling pathway leading to apoptosis involves transmembrane death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily. Members of this receptor family bind to extrinsic ligands and transduce intracellular signals that ultimately result in the destruction of the cell. The most well characterized ligands of these receptors to date are FasL, TNFα, Apo2L, and Apo3L. Corresponding receptors are FasR, TNFR1, DR3, and DR4/DR5. Molecules that stimulate the activity of these pro-apoptotic proteins or activate these receptors are currently under evaluation for their therapeutic potential in the treatment of cancer, including hematologic malignancies (Elmore, 2007. Toxicol Pathol. 35(4):495-516 (PMID: 17562483)). These extrinsic ligands are further especially preferred examples for use in the inventive approach.

New molecular insights have inspired the development of pro-apoptotic receptor agonists (PARAs), including the recombinant human protein apoptosis ligand 2/TNF-related apoptosis-inducing ligand (Apo2L/TRAIL). In addition, agonistic monoclonal antibodies to its signalling receptors DR4 (TRAILR1) and DR5 (TRAILR2) are under development. Mapatumumab is an example of a DR4

agonist antibody. Examples of DR5 agonistic antibodies include Lexatumumab, Apomab, AMG655, CS-1008 and LBY-135 (Ashkenazi, 2008. Nat. Rev. Drug Discov. 7(12):1001-12 (PMID: 18989337)).

The following table 5 summarizes some preferred apoptosis inducers.

Table 5: Apoptosis inducers

Gene/Agent	Example
Intrinsic pathway	
Bcl-10	
Bax	
Bak	
Bid	
Bad	
Bim	
Bik	
Blk	
Cytochrome c	
Caspase 3, 6, 7, 8, 9	
Extrinsic pathway	
FasL	
TNFα	
Apo2L/TRAIL	
Apo3L	
DR4 agonist antibody	Mapatumumab
DR5 agonist antibody	Lexatumumab, Apomab, AMG655, CS-1008, LBY-135
Other	
Granzyme B	

According to preferred embodiments in the context of the present invention apoptosis inducers may be selected from any apoptosis inducer selected from the group consisting of Apo2L/TRAIL; Apo3L; Bad; Bak; Bax; Bcl-10; Bid; Bik; Bim; Blk; Caspase_3; Caspase_6; Caspase_7; Caspase_8; Caspase_9; Cytochrome_c; FasL; Granzyme_B; TNF, preferably as disclosed in Table 6. Particularly preferred in this context are the RNA sequences encoding an apoptosis inducer according to Table 6.

Table 6: Apoptosis inducers:

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
Apo2L/TRAIL	UniProtKB: P50591	6897	6898	6899, 6900, 6901, 6902, 6903, 6904
Apo3L	UniProtKB: O43508	6905	6906	6907, 6908, 6909, 6910, 6911, 6912
Bad	UniProtKB: A0A024R562	6913	6914	6915, 6916, 6917, 6918, 6919, 6920
Bad	UniProtKB: Q92934	6921	6922	6923, 6924, 6925, 6926, 6927, 6928
Bak	UniProtKB: Q16611	6929	6930	6931, 6932, 6933, 6934, 6935, 6936
Bak	UniProtKB: Q8NFF3	6937	6938	6939, 6940, 6941, 6942, 6943, 6944
Bax	UniProtKB: A0A0C4MVT1	6945	6946	6947, 6948, 6949, 6950, 6951, 6952
Bax	UniProtKB: A0A0C4MW46	6953	6954	6955, 6956, 6957, 6958, 6959, 6960
Bax	UniProtKB: A0A0C4MWS3	6961	6962	6963, 6964, 6965, 6966, 6967, 6968
Bax	UniProtKB: I6LPK7	6969	6970	6971, 6972, 6973, 6974, 6975, 6976
Bax	UniProtKB: K4JQN1	6977	6978	6979, 6980, 6981, 6982, 6983, 6984
Bax	UniProtKB: Q07812	6985	6986	6987, 6988, 6989, 6990, 6991, 6992
Bcl-10	UniProtKB: O95999	6993	6994	6995, 6996, 6997, 6998, 6999, 7000
Bid	UniProtKB: A8ASI8	7001	7002	7003, 7004, 7005, 7006, 7007, 7008
Bid	UniProtKB: B2ZP78	7009	7010	7011, 7012, 7013, 7014, 7015, 7016
Bid	UniProtKB: B2ZP79	7017	7018	7019, 7020, 7021, 7022, 7023, 7024
Bid	UniProtKB: P55957	7025	7026	7027, 7028, 7029, 7030, 7031, 7032
Bik	UniProtKB: A0A024R4X6	7033	7034	7035, 7036, 7037, 7038, 7039, 7040
Bik	UniProtKB: Q13323	7041	7042	7043, 7044, 7045, 7046, 7047, 7048
Bim	UniProtKB: O43521	7049	7050	7051, 7052, 7053, 7054, 7055, 7056

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
Blk	UniProtKB: P51451	7057	7058	7059, 7060, 7061, 7062, 7063, 7064
Caspase_3	UniProtKB: P42574	7065	7066	7067, 7068, 7069, 7070, 7071, 7072
Caspase_6	UniProtKB: P55212	7073	7074	7075, 7076, 7077, 7078, 7079, 7080
Caspase_7	UniProtKB: P55210	7081	7082	7083, 7084, 7085, 7086, 7087, 7088
Caspase_8	UniProtKB: B5BU46	7089	7090	7091, 7092, 7093, 7094, 7095, 7096
Caspase_8	UniProtKB: B6CGU5	7097	7098	7099, 7100, 7101, 7102, 7103, 7104
Caspase_8	UniProtKB: C3S3G0	7105	7106	7107, 7108, 7109, 7110, 7111, 7112
Caspase_8	UniProtKB: Q14790	7113	7114	7115, 7116, 7117, 7118, 7119, 7120
Caspase_9	UniProtKB: A0A024R8F1	7121	7122	7123, 7124, 7125, 7126, 7127, 7128
Caspase_9	UniProtKB: A0A024R8I4	7129	7130	7131, 7132, 7133, 7134, 7135, 7136
Caspase_9	UniProtKB: P55211	7137	7138	7139, 7140, 7141, 7142, 7143, 7144
Caspase_9	UniProtKB: Q9H257	7145	7146	7147, 7148, 7149, 7150, 7151, 7152
Cytochrome_c	UniProtKB: A0A024R9B7	7153	7154	7155, 7156, 7157, 7158, 7159, 7160
Cytochrome_c	UniProtKB: A0A024RAP6	7161	7162	7163, 7164, 7165, 7166, 7167, 7168
Cytochrome_c	UniProtKB: A0A024RBN6	7169	7170	7171, 7172, 7173, 7174, 7175, 7176
Cytochrome_c	UniProtKB: A0A024RBY9	7177	7178	7179, 7180, 7181, 7182, 7183, 7184
Cytochrome_c	UniProtKB: B8XYC5	7185	7186	7187, 7188, 7189, 7190, 7191, 7192
Cytochrome_c	UniProtKB: G4XXL9	7193	7194	7195, 7196, 7197, 7198, 7199, 7200
Cytochrome_c	UniProtKB: H0UI06	7201	7202	7203, 7204, 7205, 7206, 7207, 7208
Cytochrome_c	UniProtKB: H6SG12	7209	7210	7211, 7212, 7213, 7214, 7215, 7216
Cytochrome_c	UniProtKB: H6SG13	7217	7218	7219, 7220, 7221, 7222,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				7223, 7224
Cytochrome_c	UniProtKB: H6SG14	7225	7226	7227, 7228, 7229, 7230, 7231, 7232
Cytochrome_c	UniProtKB: H6SG15	7233	7234	7235, 7236, 7237, 7238, 7239, 7240
Cytochrome_c	UniProtKB: O95101	7241	7242	7243, 7244, 7245, 7246, 7247, 7248
Cytochrome_c	UniProtKB: P08574	7249	7250	7251, 7252, 7253, 7254, 7255, 7256
Cytochrome_c	UniProtKB: P99999	7257	7258	7259, 7260, 7261, 7262, 7263, 7264
Cytochrome_c	UniProtKB: Q496I0	7265	7266	7267, 7268, 7269, 7270, 7271, 7272
Cytochrome_c	UniProtKB: Q53XN1	7273	7274	7275, 7276, 7277, 7278, 7279, 7280
Cytochrome_c	UniProtKB: Q6FGA0	7281	7282	7283, 7284, 7285, 7286, 7287, 7288
Cytochrome_c	UniProtKB: Q6FGI7	7289	7290	7291, 7292, 7293, 7294, 7295, 7296
Cytochrome_c	UniProtKB: Q71U45	7297	7298	7299, 7300, 7301, 7302, 7303, 7304
Cytochrome_c	UniProtKB: Q86WV2	7305	7306	7307, 7308, 7309, 7310, 7311, 7312
Cytochrome_c	UniProtKB: Q9UEG9	7313	7314	7315, 7316, 7317, 7318, 7319, 7320
FasL	UniProtKB: P48023	7321	7322	7323, 7324, 7325, 7326, 7327, 7328
Granzyme_B	UniProtKB: J3KQ52	7329	7330	7331, 7332, 7333, 7334, 7335, 7336
Granzyme_B	UniProtKB: Q67BC3	7337	7338	7339, 7340, 7341, 7342, 7343, 7344
Granzyme_B	UniProtKB: Q6XGZ2	7345	7346	7347, 7348, 7349, 7350, 7351, 7352
Granzyme_B	UniProtKB: Q6XGZ3	7353	7354	7355, 7356, 7357, 7358, 7359, 7360
Granzyme_B	UniProtKB: Q6XGZ4	7361	7362	7363, 7364, 7365, 7366, 7367, 7368
TNF	UniProtKB: P01375	7369	7370	7371, 7372, 7373, 7374, 7375, 7376
TNF	UniProtKB: Q5STB3	7377	7378	7379, 7380, 7381, 7382, 7383, 7384

According to the present invention, in a more preferred embodiment, the inventive composition comprises at least one RNA, preferably an mRNA comprising at least one coding region encoding at least one apoptosis inducer or cell death inducer or a fragment or variant thereof, wherein the at least one coding region comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequences according to the SEQ ID Nos as disclosed in Table 6.

6. Angiogenesis inhibitors

In a further preferred embodiment of the inventive RNA containing composition the at least one RNA, preferably mRNA codes for at least one angiogenesis modulator or inhibitor, preferably an endogenous angiogenesis inhibitor or a fragment or variant thereof. Tumor growth and survival depend on angiogenesis to provide a path for delivery of oxygen and nutrients to tumor cells. By using RNA coding for at least one angiogenesis inhibitor according to the inventive approach it is possible to block angiogenesis in a localized manner, namely within the tumor tissue, thereby providing an effective method for stopping tumor growth and decreasing tumor volume. Preferred examples of angiogenesis inhibitors according to the invention may be chosen from the following list: interferon alpha (IFN-α), (interferon beta) IFN-β, interferon gamma (IFN-γ), CXCL9, CXCL10, interleukin 12 (IL-12), platelet factor 4 (PF-4), tumor necrosis factor alpha (TNF-α), soluble fms-like tyrosine kinase 1 (sFLT-1), Fetal Liver Kinase 1 (FLK-1), Angiostatin, Endostatin, Vasostatin, Canstatin, Tumstatin, 16 kD prolacin fragment, tissue inhibitor of metalloproteinases 1 (TIMP-1), tissue inhibitor of metalloproteinases 2 (TIMP-2), tissue inhibitor of metalloproteinases 3 (TIMP-3), thrombospondin 1 (TSP-1), thrombospondin 2 (TSP-2), Maspin, PEX, soluble Tyrosine-protein kinase receptor 1 (sTie1), soluble Angiopoietin-1 receptor 2 (sTie2), Angiopoietin-1, Angiopoietin-2, Antivascular endothelial growth factor receptor 2 (VEGFR2) antibody (e.g. Alacizumab, Ramucirumab), Anti-vascular endothelial growth factor (VEGF) antibody (e.g. Brolucizumab, Ranibizumab, Bevacizumab), and Anti-vascular endothelial growth factor receptor 1 (VEGFR1) antibody (e.g. Icrucumab).

Without this process of blood vessel recruitment, tumor growth is limited to 1 to 2 mm^2, the diffusion limit of oxygen. Already in 1971, Folkman proposed that tumor growth could be arrested by blocking angiogenesis (Folkman, 1972. N. Engl. J. Med. 285(21):1182-6).

Angiogenesis is a multistep process of new blood vessel formation from preexisting vasculature that includes the activation, proliferation and migration of endothelial cells (ECs), disruption of vascular basement membranes, remodeling of the extracellular matrix of tissues, formation of vascular tubes and networks, recruitment of supporting cells, including smooth muscle cells and pericytes, and connection to the pre-existing vascular network.

Within a given microenvironment, the angiogenic response results from a balance between pro-angiogenic and anti-angiogenic factors, secreted both by tumor cells and components of the stroma; the prevalence of the former determines the "angiogenic switch", resulting in the activation of angiogenesis followed by tumor outgrowth (Hanahan and Folkman, 1996. Cell 86(3):353-64).

Gene therapy based strategies of angiogenesis inhibition and especially the approach according to the present invention have several advantages compared with conventional modalities of administration of anti-angiogenic drugs. First of all, since effective suppression of pathological angiogenesis may eventually require chronic treatment, the gene therapy strategy according to the invention is useful to achieve selective delivery to affected tissues and prolonged expression of the therapeutic agents. Gene therapy in general also represents a method for circumventing the production problems of many recombinant proteins including their stability and solubility; adequate production of anti-angiogenic factors by recombinant engineering methods has been sometimes problematic (e.g. for angiostatin) and may limit their clinical application. Moreover gene transfer usage allows the correct folding of proteic agents and their stability *in vivo* since they are assembled in their physiologic environment. A particularly attractive feature of the inventive approach is the possibility of targeting gene delivery to selective tissues, namely tumor tissue, thus achieving localized gene expression and high regional drug concentrations without increasing the systemic levels of the therapeutic agents and thereby resulting in an improved therapeutic index.

Angiogenesis inhibitors are heterogeneous in origin and potency, and their growing list includes proteolysis products of larger molecules with a different function, such as angiostatin, endostatin and vasostatin, modulators of vascular endothelial growth factor activity, such as soluble FLT-1 (sFLT-1), and some cytokines/chemokines with marked anti-endothelial activity, such as IL-12, IFN-

α, and CXCL10. The following table 8 (adapted from Persano et al., 2007. Mol. Aspects Med. 28(1):87-114. PMID: 17306361) summarizes the preferred angiogenesis inhibitors which may be used in the inventive approach.

According to preferred embodiments in the context of the present invention angiogenesis inhibitors may be selected from any endogenous angiogenesis inhibitor selected from the group consisting of Angiopoietin-2; Angiostatin; Canstatin; CXCL10; CXCL4; CXCL9; Endostatin; FLK-1; IFNalpha; IFNB; IFNG; IL-12; PEX; PRL; SERPINB5; sFLT-1; sTie2; TIMP-1; TIMP-2; TIMP-3; TNF; TSP-1; TSP-2; Tumstatin; Vasostatin, preferably as disclosed in Table 7. Particularly preferred in this context are the RNA sequences encoding an angiogenesis inhibitor according to Table 7.

Table 7: Endogenous angiogenesis inhibitors

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
IFNalpha	UniProtKB: G9JKF1	3953	3954	3955, 3956, 3957, 3958, 3959, 3960
IFNalpha	UniProtKB: P01562	3961	3962	3963, 3964, 3965, 3966, 3967, 3968
IFNalpha	UniProtKB: P01563	3969	3970	3971, 3972, 3973, 3974, 3975, 3976
IFNalpha	UniProtKB: P01566	3977	3978	3979, 3980, 3981, 3982, 3983, 3984
IFNalpha	UniProtKB: P01567	3985	3986	3987, 3988, 3989, 3990, 3991, 3992
IFNalpha	UniProtKB: P01568	3993	3994	3995, 3996, 3997, 3998, 3999, 4000
IFNalpha	UniProtKB: P01569	4001	4002	4003, 4004, 4005, 4006, 4007, 4008
IFNalpha	UniProtKB: P01570	4009	4010	4011, 4012, 4013, 4014, 4015, 4016
IFNalpha	UniProtKB: P01571	4017	4018	4019, 4020, 4021, 4022, 4023, 4024
IFNalpha	UniProtKB: P05013	4025	4026	4027, 4028, 4029, 4030, 4031, 4032
IFNalpha	UniProtKB: P05014	4033	4034	4035, 4036, 4037, 4038, 4039, 4040
IFNalpha	UniProtKB: P05015	4041	4042	4043, 4044, 4045, 4046, 4047, 4048

IFNalpha	UniProtKB: P32881	4049	4050	4051, 4052, 4053, 4054, 4055, 4056
IFNalpha	UniProtKB: Q14618	4057	4058	4059, 4060, 4061, 4062, 4063, 4064
IFNalpha	UniProtKB: Q86UP4	4065	4066	4067, 4068, 4069, 4070, 4071, 4072
IFNB	UniProtKB: P01574	4073	4074	4075, 4076, 4077, 4078, 4079, 4080
IFNB	UniProtKB: Q15943	4081	4082	4083, 4084, 4085, 4086, 4087, 4088
IFNG	UniProtKB: P01579	4089	4090	4091, 4092, 4093, 4094, 4095, 4096
IFNG	UniProtKB: Q14609	4097	4098	4099, 4100, 4101, 4102, 4103, 4104
IFNG	UniProtKB: Q14610	4105	4106	4107, 4108, 4109, 4110, 4111, 4112
IFNG	UniProtKB: Q14611	4113	4114	4115, 4116, 4117, 4118, 4119, 4120
IFNG	UniProtKB: Q14612	4121	4122	4123, 4124, 4125, 4126, 4127, 4128
IFNG	UniProtKB: Q14613	4129	4130	4131, 4132, 4133, 4134, 4135, 4136
IFNG	UniProtKB: Q14614	4137	4138	4139, 4140, 4141, 4142, 4143, 4144
IFNG	UniProtKB: Q14615	4145	4146	4147, 4148, 4149, 4150, 4151, 4152
IFNG	UniProtKB: Q8NHY9	4153	4154	4155, 4156, 4157, 4158, 4159, 4160
IL-12	UniProtKB: P29460	4193	4194	4195, 4196, 4197, 4198, 4199, 4200
CXCL10	UniProtKB: A0A024RDA4	5129	5130	5131, 5132, 5133, 5134, 5135, 5136
CXCL10	UniProtKB: P02778	5137	5138	5139, 5140, 5141, 5142, 5143, 5144
CXCL4	UniProtKB: P02776	5225	5226	5227, 5228, 5229, 5230, 5231, 5232
CXCL9	UniProtKB: L8E8X0	5273	5274	5275, 5276, 5277, 5278, 5279, 5280
CXCL9	UniProtKB: Q07325	5281	5282	5283, 5284, 5285, 5286, 5287, 5288
TNF	UniProtKB: P01375	7369	7370	7371, 7372, 7373, 7374, 7375, 7376
TNF	UniProtKB: Q5STB3	7377	7378	7379, 7380, 7381, 7382, 7383, 7384
Angiopoietin-2	UniProtKB: B2R6E3	7385	7386	7387, 7388, 7389, 7390, 7391, 7392

Angiopoietin-2	UniProtKB: O15123	7393	7394	7395, 7396, 7397, 7398, 7399, 7400
Angiostatin	UniProtKB: A0A0F7G8J1	7401	7402	7403, 7404, 7405, 7406, 7407, 7408
Angiostatin	UniProtKB: P00747	7409	7410	7411, 7412, 7413, 7414, 7415, 7416
Angiostatin	UniProtKB: Q5TEH5	7417	7418	7419, 7420, 7421, 7422, 7423, 7424
Canstatin	UniProtKB: P08572	7425	7426	7427, 7428, 7429, 7430, 7431, 7432
Endostatin	Homo_sapiens	7433	7434	7435, 7436, 7437, 7438, 7439, 7440
FLK-1	UniProtKB: P35968	7441	7442	7443, 7444, 7445, 7446, 7447, 7448
PEX	UniProtKB: P78562	7449	7450	7451, 7452, 7453, 7454, 7455, 7456
PRL	UniProtKB: P01236	7457	7458	7459, 7460, 7461, 7462, 7463, 7464
SERPINB5	UniProtKB: P36952	7465	7466	7467, 7468, 7469, 7470, 7471, 7472
sFLT-1	UniProtKB: H9N1E7	7473	7474	7475, 7476, 7477, 7478, 7479, 7480
sFLT-1	UniProtKB: H9N1E8	7481	7482	7483, 7484, 7485, 7486, 7487, 7488
sFLT-1	UniProtKB: L7RSL3	7489	7490	7491, 7492, 7493, 7494, 7495, 7496
sFLT-1	UniProtKB: P17948	7497	7498	7499, 7500, 7501, 7502, 7503, 7504
sTie2	UniProtKB: B5A953	7505	7506	7507, 7508, 7509, 7510, 7511, 7512
TIMP-1	UniProtKB: P01033	7513	7514	7515, 7516, 7517, 7518, 7519, 7520
TIMP-2	UniProtKB: P16035	7521	7522	7523, 7524, 7525, 7526, 7527, 7528
TIMP-3	UniProtKB: P35625	7529	7530	7531, 7532, 7533, 7534, 7535, 7536
TSP-1	UniProtKB: P07996	7537	7538	7539, 7540, 7541, 7542, 7543, 7544
TSP-2	UniProtKB: P35442	7545	7546	7547, 7548, 7549, 7550, 7551, 7552
Tumstatin	UniProtKB: Q01955	7553	7554	7555, 7556, 7557, 7558, 7559, 7560
Vasostatin	UniProtKB: P10645	7561	7562	7563, 7564, 7565, 7566, 7567, 7568

According to the present invention, in a more preferred embodiment, the inventive composition comprises at least one RNA, preferably an mRNA comprising at least one coding region encoding at least one angiogenesis inhibitor or a fragment or variant thereof, wherein the at least one coding region comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequences according to the SEQ ID Nos as disclosed in Table 7.

7. Heat shock proteins

In a further preferred embodiment of the inventive RNA containing composition the RNA codes for at least one heat shock protein (HSP) or a fragment or variant thereof. Preferably, the heat shock protein may be chosen from the following list: HSP27, HSP47 (serpin H1), HSP60, HSP70, HSC70, GRP78 (BiP), HSP90, HSP110, GRP94 (gp96), GRP170 (ORP150), PDI/PDIA, CRT/CALR.

As reviewed by Graner et al. (Graner MW, Lillehei KO, Katsanis E. Endoplasmic reticulum chaperones and their roles in the immunogenicity of cancer vaccines. Front Oncol. 2015 Jan 6; 4:379. doi: 10.3389/fonc.2014.00379) heat shock proteins play essential cellular housekeeping functions and are indispensible during protein synthesis, folding and transport across intracellular membranes as well as protein degradation. HSPs belong to a multiprotein family of chaperons which consists of, but is not limited to, HSP27, HSP47 (serpin H1), HSP60, HSP70, HSC70, GRP78 (BiP), HSP90, HSP110, GRP94 (gp96), GRP170 (ORP150), PDI/PDIA, CRT/CALR. In addition to the intracellular functions as chaperons, HSPs have been shown to play an important extracellular role as simulators of the immune responses particularly in tumor settings. Various literature reports demonstrated that tumor-derived HSP-peptide complexes induce anti-tumor immune responses very efficiently. The molecular mechanism of these observations has been elucidated. HSPs as chaperons have the capacity to bind denatured peptides including the antigenic ones and those complexes are internalized by antigen presenting cells (APCs) which eventually leads to antigen presentation and induction of immunity. In addition to their chaperon function, HSPs have been shown to trigger danger signals in the tumor microenvironment and thus stimulate macrophages and dendritic cells (DCs) to produce proinflammatory cytokines and enhance the induced immune responses.

According to preferred embodiments in the context of the present invention heat shock proteins may be selected from any heat shock protein selected from the group consisting of calreticulin; GRP170_(ORP150); GRP78_(BiP); GRP94_(gp96); HSC70; HSP110; HSP27; HSP47_(serpin_H1); HSP60; HSP70; HSP90; PDI/PDIA, preferably as disclosed in Table 8. Particularly preferred in this context are the RNA sequences encoding a heat shock protein according to Table 8.

Table 8: Heat shock proteins

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
calreticulin	UniProtKB: B4DHR1	7569	7570	7571, 7572, 7573, 7574, 7575, 7576
calreticulin	UniProtKB: B4E2Y9	7577	7578	7579, 7580, 7581, 7582, 7583, 7584
calreticulin	UniProtKB: P27797	7585	7586	7587, 7588, 7589, 7590, 7591, 7592
calreticulin	UniProtKB: Q96L12	7593	7594	7595, 7596, 7597, 7598, 7599, 7600
GRP170_(ORP150)	UniProtKB: Q9Y4L1	7601	7602	7603, 7604, 7605, 7606, 7607, 7608
GRP78_(BiP)	UniProtKB: P11021	7609	7610	7611, 7612, 7613, 7614, 7615, 7616
GRP94_(gp96)	UniProtKB: P14625	7617	7618	7619, 7620, 7621, 7622, 7623, 7624
HSC70	UniProtKB: P11142	7625	7626	7627, 7628, 7629, 7630, 7631, 7632
HSP110	UniProtKB: Q92598	7633	7634	7635, 7636, 7637, 7638, 7639, 7640
HSP27	UniProtKB: P04792	7641	7642	7643, 7644, 7645, 7646, 7647, 7648
HSP47_(serpin_H1)	UniProtKB: P50454	7649	7650	7651, 7652, 7653, 7654, 7655, 7656
HSP60	UniProtKB: A0A024R3X4	7657	7658	7659, 7660, 7661, 7662, 7663, 7664
HSP60	UniProtKB: B3GQS7	7665	7666	7667, 7668, 7669, 7670, 7671, 7672
HSP60	UniProtKB: P10809	7673	7674	7675, 7676, 7677, 7678, 7679, 7680
HSP60	UniProtKB: Q0VDF9	7681	7682	7683, 7684, 7685, 7686, 7687, 7688
HSP70	UniProtKB: P38646	7689	7690	7691, 7692, 7693, 7694,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				7695, 7696
HSP90	UniProtKB: P07900	7697	7698	7699, 7700, 7701, 7702, 7703, 7704
HSP90	UniProtKB: P08238	7705	7706	7707, 7708, 7709, 7710, 7711, 7712
PDI/PDIA	UniProtKB: P07237	7713	7714	7715, 7716, 7717, 7718, 7719, 7720
PDI/PDIA	UniProtKB: Q6YPB0	7721	7722	7723, 7724, 7725, 7726, 7727, 7728
PDI/PDIA	UniProtKB: Q71S60	7729	7730	7731, 7732, 7733, 7734, 7735, 7736

According to the present invention, in a more preferred embodiment, the inventive composition comprises at least one RNA, preferably an mRNA comprising at least one coding region encoding at least one heat shock protein or a fragment or variant thereof, wherein the at least one coding region comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequences according to the SEQ ID Nos as disclosed in Table 8.

8. Tumor antigens

In a further preferred embodiment of the inventive RNA containing composition the composition may contain RNA, preferably mRNA which codes for at least one tumor antigen or a fragment or variant thereof, which are used for vaccination to induce an adaptive immune response according to the invention.

In this context tumor antigens are particularly preferred to be encoded by RNA, preferably mRNA comprised in the inventive RNA composition. It is particularly preferred that the inventive RNA composition comprises at least one RNA encoding at least one tumor antigen or a fragment or variant thereof.

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) degenerated 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.

Further, tumor associated antigens may be classified as tissue-specific antigens, also called melanocyte-specific antigens, cancer-testis antigens and tumor-specific antigens. Cancer-testis antigens are typically understood to be peptides or proteins of germ-line associated genes which may be activated in a wide variety of tumors. Human cancer-testis antigens may be further subdivided into antigens which are encoded on the X chromosome, so-called CT-X antigens, and those antigens which are not encoded on the X chromosome, the so-called (non-X CT antigens). Cancer-testis antigens which are encoded on the X-chromosome comprises, for example, the family of melanoma antigen genes, the so-called MAGE-family. The genes of the MAGE-family may be characterised by a shared MAGE homology domain (MHD). Each of these antigens, i.e. melanocyte-specific antigens, cancer-testis antigens and tumor-specific antigens, may elicit autologous cellular and humoral immune response. Accordingly, the tumor antigen encoded by the inventive nucleic acid sequence is preferably a melanocyte-specific antigen, a cancer-testis antigen or a tumor-specific antigens, preferably it may be a CT-X antigen, a non-X CT-antigens, a binding partner for a CT-X antigen or a binding partner for a non-X CT-antigen or a tumor-specific antigen , more preferably a CT-X antigen, a binding partner for a non-X CT-antigen or a tumor-specific antigen.

Particular preferred tumor antigens are selected from the list 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-B, NY-ESO-1, 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, PAP, 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-1, 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, VEGFR1, VEGFR-2/FLK-1, and WT1. Such tumor antigens preferably may be selected from the group consisting of p53, CA125, EGFR, Her2/neu, hTERT, PAP, MAGE-A1, MAGE-A3, Mesothelin, MUC-1, GP100, MART-1, Tyrosinase, PSA, PSCA, PSMA, STEAP-1, VEGF, VEGFR1, VEGFR2, Ras, CEA or WT1, and more preferably from PAP, MAGE-A3, WT1, and MUC-1. Such tumor antigens preferably may be 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), surviving (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), TRP-2 (e.g. TRP-2 according to accession number NM_001922), STEAP1, PCA, PSA, PSMA, etc.

According to preferred embodiments in the context of the present invention tumor antigens may be selected from any tumor antigen selected from the group consisting of 1A01_HLA-A/m; 1A02; 5T4; ACRBP; AFP; AKAP4; alpha-actinin-_4/m; alpha-methylacyl-coenzyme_A_racemase; ANDR; ART-4; ARTC1/m; AURKB; B2MG; B3GN5; B4GN1; B7H4; BAGE-1; BASI; BCL-2; bcr/abl; beta-catenin/m; BING-4; BIRC7; BRCA1/m; BY55; calreticulin; CAMEL; CASPA; Caspase_8; cathepsin_B; cathepsin_L; CD1A; CD1B; CD1C; CD1D; CD1E; CD20; CD22; CD276; CD33; CD3E; CD3Z; CD4; CD44_Isoform_1; CD44_Isoform_6; CD52; CD55; CD56; CD80; CD86; CD8A; CDC27/m; CDE30; CDK4/m; CDKN2A/m; CEA; CEAM6; CH3L2; CLCA2; CML28; CML66; COA-1/m; coactosin-like_protein; collagen_XXIII; COX-2; CP1B1; CSAG2; CT-_9/BRD6; CT45A1; CT55; CTAG2_Isoform_LAGE-1A; CTAG2_Isoform_LAGE-1B; CTCFL; Cten; cyclin_B1; cyclin_D1; cyp-B; DAM-10; DEP1A; E7; EF1A2; EFTUD2/m; EGFR; EGLN3; ELF2/m; EMMPRIN; EpCam; EphA2; EphA3; ErbB3; ERBB4; ERG; ETV6; EWS; EZH2; FABP7; FCGR3A_Version_1; FCGR3A_Version_2; FGF5; FGFR2; fibronectin; FOS; FOXP3; FUT1; G250; GAGE-1; GAGE-2; GAGE-3; GAGE-4; GAGE-5; GAGE-6; GAGE7b; GAGE-8_(GAGE-2D); GASR; GnT-V; GPC3; GPNMB/m; GRM3; HAGE; hepsin; Her2/neu; HLA-A2/m; homeobox_NKX3.1; HOM-TES-85; HPG1; HS71A; HS71B; HST-2; hTERT; iCE; IF2B3; IL-10; IL-13Ra2; IL2-RA; IL2-RB; IL2-RG; IL-5; IMP3; ITA5; ITB1; ITB6; kallikrein-2; kallikrein-4; KI20A; KIAA0205; KIF2C; KK-LC-1; LDLR; LGMN; LIRB2; LY6K; MAGA5; MAGA8; MAGAB; MAGE-_B1; MAGE-_E1; MAGE-A1; MAGE-A10; MAGE-A12; MAGE-A2; MAGE-A3; MAGE-A4; MAGE-A6; MAGE-A9; MAGE-B10; MAGE-B16; MAGE-B17; MAGE-B2; MAGE-B3; MAGE-B4; MAGE-B5; MAGE-B6; MAGE-C1; MAGE-C2; MAGE-C3; MAGE-D1; MAGE-D2; MAGE-D4; MAGE-E1_(MAGE1); MAGE-E2; MAGE-

F1; MAGE-H1; MAGEL2; mammaglobin_A; MART-1/melan-A; MART-2; MC1_R; M-CSF; mesothelin; MITF; MMP1_1; MMP7; MUC-1; MUM-1/m; MUM-2/m; MYO1A; MYO1B; MYO1C; MYO1D; MYO1E; MYO1F; MYO1G; MYO1H; NA17; NA88-A; Neo-PAP; NFYC/m; NGEP; N-myc; NPM; NRCAM; NSE; NUF2; NY-ESO-1; OA1; OGT; OS-9; osteocalcin; osteopontin; p53; PAGE-4; PAI-1; PAI-2; PAP; PATE; PAX3; PAX5; PD1L1; PDCD1; PDEF; PECA1; PGCB; PGFRB; Pim-1_-Kinase; Pin-1; PLAC1; PMEL; PML; POTE; POTEF; PRAME; PRDX5/m; PRM2; prostein; proteinase-3; PSA; PSB9; PSCA; PSGR; PSM; PTPRC; RAB8A; RAGE-1; RARA; RASH; RASK; RASN; RGS5; RHAMM/CD168; RHOC; RSSA; RU1; RU2; RUNX1; S-100; SAGE; SART-_1; SART-2; SART-3; SEPR; SERPINB5; SIA7F; SIA8A; SIAT9; SIRT2/m; SOX10; SP17; SPNXA; SPXN3; SSX-1; SSX-2; SSX3; SSX-4; ST1A1; STAG2; STAMP-1; STEAP-1; survivin; Survivin-2B; SYCP1; SYT-SSX-1; SYT-SSX-2; TARP; TCRg; TF2AA; TGFbeta1; TGFR2; TGM-4; TIE2; TKTL1; TPI/m; TRGV11; TRGV9; TRPC1; TRP-p8; TSG10; TSPY1; TVC_(TRGV3); TX101; tyrosinase; TYRP1; TYRP2; UPA; VEGFR1; WT1; XAGE1, preferably as disclosed in Table 9. Particularly preferred in this context are the RNA sequences encoding a tumor antigen according to Table 9.

Table 9: Tumor antigens

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
1A01_HLA-A/m	UniProtKB: P30443	398	399	400, 401, 402, 403, 404
1A02	UniProtKB: P01892	405	406	407, 408, 409, 410, 411
5T4	UniProtKB: Q13641	412	413	414, 415, 416, 417, 418
ACRBP	UniProtKB: Q8NEB7	419	420	421, 422, 423, 424, 425
AFP	UniProtKB: P02771	426	427	428, 429, 430, 431, 432
AKAP4	UniProtKB: Q5JQC9	433	434	435, 436, 437, 438, 439
alpha-actinin-_4/m	UniProtKB: B4DSX0	440	441	442, 443, 444, 445, 446
alpha-actinin-_4/m	UniProtKB: B4E337	447	448	449, 450, 451, 452, 453
alpha-actinin-_4/m	UniProtKB: O43707	454	455	456, 457, 458, 459, 460
alpha-methylacyl-coenzyme_A_racemase	UniProtKB: A0A024RE16	461	462	463, 464, 465, 466, 467
alpha-methylacyl-coenzyme_A_racemase	UniProtKB: A8KAC3	468	469	470, 471, 472, 473, 474
ANDR	UniProtKB: P10275	475	476	477, 478, 479, 480, 481
ART-4	UniProtKB: Q9ULX3	482	483	484, 485, 486, 487, 488
ARTC1/m	UniProtKB: P52961	489	490	491, 492, 493, 494, 495
AURKB	UniProtKB: Q96GD4	496	497	498, 499, 500, 501, 502

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
B2MG	UniProtKB: P61769	503	504	505, 506, 507, 508, 509
B3GN5	UniProtKB: Q9BYG0	510	511	512, 513, 514, 515, 516
B4GN1	UniProtKB: Q00973	517	518	519, 520, 521, 522, 523
B7H4	UniProtKB: Q7Z7D3	524	525	526, 527, 528, 529, 530
BAGE-1	UniProtKB: Q13072	531	532	533, 534, 535, 536, 537
BASI	UniProtKB: P35613	538	539	540, 541, 542, 543, 544
BCL-2	UniProtKB: A9QXG9	545	546	547, 548, 549, 550, 551
bcr/abl	UniProtKB: A9UEZ4	552	553	554, 555, 556, 557, 558
bcr/abl	UniProtKB: A9UEZ7	559	560	561, 562, 563, 564, 565
bcr/abl	UniProtKB: A9UEZ8	566	567	568, 569, 570, 571, 572
bcr/abl	UniProtKB: A9UEZ9	573	574	575, 576, 577, 578, 579
bcr/abl	UniProtKB: A9UF00	580	581	582, 583, 584, 585, 586
bcr/abl	UniProtKB: A9UF01	587	588	589, 590, 591, 592, 593
bcr/abl	UniProtKB: A9UF03	594	595	596, 597, 598, 599, 600
bcr/abl	UniProtKB: A9UF04	601	602	603, 604, 605, 606, 607
bcr/abl	UniProtKB: A9UF05	608	609	610, 611, 612, 613, 614
bcr/abl	UniProtKB: A9UF06	615	616	617, 618, 619, 620, 621
bcr/abl	UniProtKB: A9UF08	622	623	624, 625, 626, 627, 628
beta-catenin/m	UniProtKB: P35222	629	630	631, 632, 633, 634, 635
beta-catenin/m	UniProtKB: Q8WYA6	636	637	638, 639, 640, 641, 642
BING-4	UniProtKB: O15213	643	644	645, 646, 647, 648, 649
BIRC7	UniProtKB: Q96CA5	650	651	652, 653, 654, 655, 656
BRCA1/m	UniProtKB: A0A024R1V0	657	658	659, 660, 661, 662, 663
BRCA1/m	UniProtKB: A0A024R1V7	664	665	666, 667, 668, 669, 670
BRCA1/m	UniProtKB: A0A024R1Z8	671	672	673, 674, 675, 676, 677
BRCA1/m	UniProtKB: A0A068BFX7	678	679	680, 681, 682, 683, 684
BRCA1/m	UniProtKB: C6YB45	685	686	687, 688, 689, 690, 691
BRCA1/m	UniProtKB: C6YB47	692	693	694, 695, 696, 697, 698
BRCA1/m	UniProtKB: G3XAC3	699	700	701, 702, 703, 704, 705
BY55	UniProtKB: O95971	706	707	708, 709, 710, 711, 712
CAMEL	UniProtKB: O95987	713	714	715, 716, 717, 718, 719
CASPA	UniProtKB: Q92851-4	720	721	722, 723, 724, 725, 726
cathepsin_B	UniProtKB: A0A024R374	727	728	729, 730, 731, 732, 733
cathepsin_B	UniProtKB: P07858	734	735	736, 737, 738, 739, 740
cathepsin_L	UniProtKB: A0A024R276	741	742	743, 744, 745, 746, 747
cathepsin_L	UniProtKB: P07711	748	749	750, 751, 752, 753, 754

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
cathepsin_L	UniProtKB: Q9HBQ7	755	756	757, 758, 759, 760, 761
CD1A	UniProtKB: P06126	762	763	764, 765, 766, 767, 768
CD1B	UniProtKB: P29016	769	770	771, 772, 773, 774, 775
CD1C	UniProtKB: P29017	776	777	778, 779, 780, 781, 782
CD1D	UniProtKB: P15813	783	784	785, 786, 787, 788, 789
CD1E	UniProtKB: P15812	790	791	792, 793, 794, 795, 796
CD20	UniProtKB: P11836	797	798	799, 800, 801, 802, 803
CD22	UniProtKB: O60926	804	805	806, 807, 808, 809, 810
CD22	UniProtKB: P20273	811	812	813, 814, 815, 816, 817
CD22	UniProtKB: Q0EAF5	818	819	820, 821, 822, 823, 824
CD276	UniProtKB: Q5ZPR3	825	826	827, 828, 829, 830, 831
CD33	UniProtKB: B4DF51	832	833	834, 835, 836, 837, 838
CD33	UniProtKB: P20138	839	840	841, 842, 843, 844, 845
CD33	UniProtKB: Q546G0	846	847	848, 849, 850, 851, 852
CD3E	UniProtKB: P07766	853	854	855, 856, 857, 858, 859
CD3Z	UniProtKB: P20963	860	861	862, 863, 864, 865, 866
CD44_Isoform_1	UniProtKB: P16070	867	868	869, 870, 871, 872, 873
CD44_Isoform_6	UniProtKB: P16070-6	874	875	876, 877, 878, 879, 880
CD4	UniProtKB: P01730	881	882	883, 884, 885, 886, 887
CD52	UniProtKB: P31358	888	889	890, 891, 892, 893, 894
CD52	UniProtKB: Q6IBD0	895	896	897, 898, 899, 900, 901
CD52	UniProtKB: V9HWN9	902	903	904, 905, 906, 907, 908
CD55	UniProtKB: B1AP15	909	910	911, 912, 913, 914, 915
CD55	UniProtKB: D3DT85	916	917	918, 919, 920, 921, 922
CD55	UniProtKB: D3DT86	923	924	925, 926, 927, 928, 929
CD55	UniProtKB: P08174	930	931	932, 933, 934, 935, 936
CD56	UniProtKB: P13591	937	938	939, 940, 941, 942, 943
CD80	UniProtKB: A0N0P2	944	945	946, 947, 948, 949, 950
CD80	UniProtKB: P33681	951	952	953, 954, 955, 956, 957
CD86	UniProtKB: P42081	958	959	960, 961, 962, 963, 964
CD8A	UniProtKB: P01732	965	966	967, 968, 969, 970, 971
CDC27/m	UniProtKB: G5EA36	972	973	974, 975, 976, 977, 978
CDC27/m	UniProtKB: P30260	979	980	981, 982, 983, 984, 985
CDE30	UniProtKB: P28908	986	987	988, 989, 990, 991, 992
CDK4/m	UniProtKB: A0A024RBB6	993	994	995, 996, 997, 998, 999
CDK4/m	UniProtKB: P11802	1000	1001	1002, 1003, 1004, 1005, 1006

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
CDK4/m	UniProtKB: Q6LC83	1007	1008	1009, 1010, 1011, 1012, 1013
CDK4/m	UniProtKB: Q96BE9	1014	1015	1016, 1017, 1018, 1019, 1020
CDKN2A/m	UniProtKB: D1LYX3	1021	1022	1023, 1024, 1025, 1026, 1027
CDKN2A/m	UniProtKB: G3XAG3	1028	1029	1030, 1031, 1032, 1033, 1034
CDKN2A/m	UniProtKB: K7PML8	1035	1036	1037, 1038, 1039, 1040, 1041
CDKN2A/m	UniProtKB: L8E941	1042	1043	1044, 1045, 1046, 1047, 1048
CDKN2A/m	UniProtKB: Q8N726	1049	1050	1051, 1052, 1053, 1054, 1055
CEA	RefSeq: NP_004354	1056	1057	1058, 1059, 1060, 1061, 1062
CEAM6	UniProtKB: P40199	1063	1064	1065, 1066, 1067, 1068, 1069
CH3L2	UniProtKB: Q15782	1070	1071	1072, 1073, 1074, 1075, 1076
CLCA2	UniProtKB: Q9UQC9	1077	1078	1079, 1080, 1081, 1082, 1083
CML28	UniProtKB: Q9NQT4	1084	1085	1086, 1087, 1088, 1089, 1090
CML66	UniProtKB: Q96RS6	1091	1092	1093, 1094, 1095, 1096, 1097
COA-1/m	UniProtKB: Q5T124	1098	1099	1100, 1101, 1102, 1103, 1104
coactosin-like_protein	UniProtKB: Q14019	1105	1106	1107, 1108, 1109, 1110, 1111
collagen_XXIII	UniProtKB: L8EAS4	1112	1113	1114, 1115, 1116, 1117, 1118
collagen_XXIII	UniProtKB: Q86Y22	1119	1120	1121, 1122, 1123, 1124, 1125
COX-2	UniProtKB: Q6ZYK7	1126	1127	1128, 1129, 1130, 1131, 1132
CP1B1	UniProtKB: Q16678	1133	1134	1135, 1136, 1137, 1138, 1139
CSAG2	UniProtKB: Q9Y5P2-2	1140	1141	1142, 1143, 1144, 1145, 1146
CSAG2	UniProtKB: Q9Y5P2	1147	1148	1149, 1150, 1151,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				1152, 1153
CT45A1	UniProtKB: Q5HYN5	1154	1155	1156, 1157, 1158, 1159, 1160
CT55	UniProtKB: Q8WUE5	1161	1162	1163, 1164, 1165, 1166, 1167
CT-_9/BRD6	UniProtKB: Q58F21	1168	1169	1170, 1171, 1172, 1173, 1174
CTAG2_Isoform_LAGE-1A	UniProtKB: O75638-2	1175	1176	1177, 1178, 1179, 1180, 1181
CTAG2_Isoform_LAGE-1B	UniProtKB: O75638	1182	1183	1184, 1185, 1186, 1187, 1188
CTCFL	UniProtKB: Q8NI51	1189	1190	1191, 1192, 1193, 1194, 1195
Cten	UniProtKB: Q8IZW8	1196	1197	1198, 1199, 1200, 1201, 1202
cyclin_B1	UniProtKB: P14635	1203	1204	1205, 1206, 1207, 1208, 1209
cyclin_D1	UniProtKB: P24385	1210	1211	1212, 1213, 1214, 1215, 1216
cyp-B	UniProtKB: P23284	1217	1218	1219, 1220, 1221, 1222, 1223
DAM-10	UniProtKB: P43366	1224	1225	1226, 1227, 1228, 1229, 1230
DEP1A	UniProtKB: Q5TB30	1231	1232	1233, 1234, 1235, 1236, 1237
E7	UniProtKB: P03129	1238	1239	1240, 1241, 1242, 1243, 1244
E7	UniProtKB: P06788	1245	1246	1247, 1248, 1249, 1250, 1251
E7	UniProtKB: P17387	1252	1253	1254, 1255, 1256, 1257, 1258
E7	UniProtKB: P06429	1259	1260	1261, 1262, 1263, 1264, 1265
E7	UniProtKB: P27230	1266	1267	1268, 1269, 1270, 1271, 1272
E7	UniProtKB: P24837	1273	1274	1275, 1276, 1277, 1278, 1279
E7	UniProtKB: P21736	1280	1281	1282, 1283, 1284, 1285, 1286
E7	UniProtKB: P26558	1287	1288	1289, 1290, 1291, 1292, 1293

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
E7	UniProtKB: P36831	1294	1295	1296, 1297, 1298, 1299, 1300
E7	UniProtKB: P36833	1301	1302	1303, 1304, 1305, 1306, 1307
E7	UniProtKB: Q9QCZ1	1308	1309	1310, 1311, 1312, 1313, 1314
E7	UniProtKB: Q81965	1315	1316	1317, 1318, 1319, 1320, 1321
E7	UniProtKB: Q80956	1322	1323	1324, 1325, 1326, 1327, 1328
EF1A2	UniProtKB: Q05639	1329	1330	1331, 1332, 1333, 1334, 1335
EFTUD2/m	UniProtKB: Q15029	1336	1337	1338, 1339, 1340, 1341, 1342
EGFR	UniProtKB: A0A0B4J1Y5	1343	1344	1345, 1346, 1347, 1348, 1349
EGFR	UniProtKB: E7BSV0	1350	1351	1352, 1353, 1354, 1355, 1356
EGFR	UniProtKB: L0R6G1	1357	1358	1359, 1360, 1361, 1362, 1363
EGFR	UniProtKB: P00533-2	1364	1365	1366, 1367, 1368, 1369, 1370
EGFR	UniProtKB: P00533	1371	1372	1373, 1374, 1375, 1376, 1377
EGFR	UniProtKB: Q147T7	1378	1379	1380, 1381, 1382, 1383, 1384
EGFR	UniProtKB: Q504U8	1385	1386	1387, 1388, 1389, 1390, 1391
EGFR	UniProtKB: Q8NDU8	1392	1393	1394, 1395, 1396, 1397, 1398
EGLN3	UniProtKB: Q9H6Z9	1399	1400	1401, 1402, 1403, 1404, 1405
ELF2/m	UniProtKB: B7Z720	1406	1407	1408, 1409, 1410, 1411, 1412
EMMPRIN	UniProtKB: Q54A51	1413	1414	1415, 1416, 1417, 1418, 1419
EpCam	UniProtKB: P16422	1420	1421	1422, 1423, 1424, 1425, 1426
EphA2	UniProtKB: P29317	1427	1428	1429, 1430, 1431, 1432, 1433
EphA3	UniProtKB: P29320	1434	1435	1436, 1437, 1438,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				1439, 1440
EphA3	UniProtKB: Q6P4R6	1441	1442	1443, 1444, 1445, 1446, 1447
ErbB3	UniProtKB: B3KWG5	1448	1449	1450, 1451, 1452, 1453, 1454
ErbB3	UniProtKB: B4DGQ7	1455	1456	1457, 1458, 1459, 1460, 1461
ERBB4	UniProtKB: Q15303	1462	1463	1464, 1465, 1466, 1467, 1468
ERG	UniProtKB: P11308	1469	1470	1471, 1472, 1473, 1474, 1475
ETV6	UniProtKB: P41212	1476	1477	1478, 1479, 1480, 1481, 1482
EWS	UniProtKB: Q01844	1483	1484	1485, 1486, 1487, 1488, 1489
EZH2	UniProtKB: F2YMM1	1490	1491	1492, 1493, 1494, 1495, 1496
EZH2	UniProtKB: G3XAL2	1497	1498	1499, 1500, 1501, 1502, 1503
EZH2	UniProtKB: L0R855	1504	1505	1506, 1507, 1508, 1509, 1510
EZH2	UniProtKB: Q15910	1511	1512	1513, 1514, 1515, 1516, 1517
EZH2	UniProtKB: S4S3R8	1518	1519	1520, 1521, 1522, 1523, 1524
FABP7	UniProtKB: O15540	1525	1526	1527, 1528, 1529, 1530, 1531
FCGR3A_Version_1	UniProtKB: P08637	1532	1533	1534, 1535, 1536, 1537, 1538
FCGR3A_Version_2	CCDS: CCDS1232.1	1539	1540	1541, 1542, 1543, 1544, 1545
FGF5	UniProtKB: P12034	1546	1547	1548, 1549, 1550, 1551, 1552
FGF5	UniProtKB: Q60518	1553	1554	1555, 1556, 1557, 1558, 1559
FGFR2	UniProtKB: P21802	1560	1561	1562, 1563, 1564, 1565, 1566
fibronectin	UniProtKB: A0A024R5I6	1567	1568	1569, 1570, 1571, 1572, 1573
fibronectin	UniProtKB: A0A024RB01	1574	1575	1576, 1577, 1578, 1579, 1580

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
fibronectin	UniProtKB: A0A024RDT9	1581	1582	1583, 1584, 1585, 1586, 1587
fibronectin	UniProtKB: A0A024RDV5	1588	1589	1590, 1591, 1592, 1593, 1594
fibronectin	UniProtKB: A6NH44	1595	1596	1597, 1598, 1599, 1600, 1601
fibronectin	UniProtKB: A8K6A5	1602	1603	1604, 1605, 1606, 1607, 1608
fibronectin	UniProtKB: B2R627	1609	1610	1611, 1612, 1613, 1614, 1615
fibronectin	UniProtKB: B3KXM5	1616	1617	1618, 1619, 1620, 1621, 1622
fibronectin	UniProtKB: B4DIC5	1623	1624	1625, 1626, 1627, 1628, 1629
fibronectin	UniProtKB: B4DN21	1630	1631	1632, 1633, 1634, 1635, 1636
fibronectin	UniProtKB: B4DS98	1637	1638	1639, 1640, 1641, 1642, 1643
fibronectin	UniProtKB: B4DTH2	1644	1645	1646, 1647, 1648, 1649, 1650
fibronectin	UniProtKB: B4DTK1	1651	1652	1653, 1654, 1655, 1656, 1657
fibronectin	UniProtKB: B4DU16	1658	1659	1660, 1661, 1662, 1663, 1664
fibronectin	UniProtKB: B7Z3W5	1665	1666	1667, 1668, 1669, 1670, 1671
fibronectin	UniProtKB: B7Z939	1672	1673	1674, 1675, 1676, 1677, 1678
fibronectin	UniProtKB: G5E9X3	1679	1680	1681, 1682, 1683, 1684, 1685
fibronectin	UniProtKB: Q9H382	1686	1687	1688, 1689, 1690, 1691, 1692
FOS	UniProtKB: P01100	1693	1694	1695, 1696, 1697, 1698, 1699
FOXP3	UniProtKB: Q9BZS1	1700	1701	1702, 1703, 1704, 1705, 1706
FUT1	UniProtKB: P19526	1707	1708	1709, 1710, 1711, 1712, 1713
G250	UniProtKB: Q16790	1714	1715	1716, 1717, 1718, 1719, 1720
GAGE-1	Genbank: AAA82744	1721	1722	1723, 1724, 1725,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				1726, 1727
GAGE-2	UniProtKB: Q6NT46	1728	1729	1730, 1731, 1732, 1733, 1734
GAGE-3	UniProtKB: Q13067	1735	1736	1737, 1738, 1739, 1740, 1741
GAGE-4	UniProtKB: Q13068	1742	1743	1744, 1745, 1746, 1747, 1748
GAGE-5	UniProtKB: Q13069	1749	1750	1751, 1752, 1753, 1754, 1755
GAGE-6	UniProtKB: Q13070	1756	1757	1758, 1759, 1760, 1761, 1762
GAGE7b	UniProtKB: O76087	1763	1764	1765, 1766, 1767, 1768, 1769
GAGE-8_(GAGE-2D)	UniProtKB: Q9UEU5	1770	1771	1772, 1773, 1774, 1775, 1776
GASR	UniProtKB: P32239	1777	1778	1779, 1780, 1781, 1782, 1783
GnT-V	UniProtKB: Q09328	1784	1785	1786, 1787, 1788, 1789, 1790
GPC3	UniProtKB: I6QTG3	1791	1792	1793, 1794, 1795, 1796, 1797
GPC3	UniProtKB: P51654	1798	1799	1800, 1801, 1802, 1803, 1804
GPC3	UniProtKB: Q8IYG2	1805	1806	1807, 1808, 1809, 1810, 1811
GPNMB/m	UniProtKB: A0A024RA55	1812	1813	1814, 1815, 1816, 1817, 1818
GPNMB/m	UniProtKB: Q14956	1819	1820	1821, 1822, 1823, 1824, 1825
GPNMB/m	UniProtKB: Q8IXJ5	1826	1827	1828, 1829, 1830, 1831, 1832
GPNMB/m	UniProtKB: Q96F58	1833	1834	1835, 1836, 1837, 1838, 1839
GRM3	UniProtKB: Q14832	1840	1841	1842, 1843, 1844, 1845, 1846
HAGE	UniProtKB: Q9NXZ2	1847	1848	1849, 1850, 1851, 1852, 1853
hepsin	UniProtKB: B2ZDQ2	1854	1855	1856, 1857, 1858, 1859, 1860
hepsin	UniProtKB: P05981	1861	1862	1863, 1864, 1865, 1866, 1867

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
Her2/neu	UniProtKB: B4DTR1	1868	1869	1870, 1871, 1872, 1873, 1874
Her2/neu	UniProtKB: L8E8G2	1875	1876	1877, 1878, 1879, 1880, 1881
Her2/neu	UniProtKB: P04626	1882	1883	1884, 1885, 1886, 1887, 1888
Her2/neu	UniProtKB: Q9UK79	1889	1890	1891, 1892, 1893, 1894, 1895
HLA-A2/m	UniProtKB: Q95387	1896	1897	1898, 1899, 1900, 1901, 1902
HLA-A2/m	UniProtKB: Q9MYF8	1903	1904	1905, 1906, 1907, 1908, 1909
homeobox_NKX3.1	UniProtKB: Q99801	1910	1911	1912, 1913, 1914, 1915, 1916
HOM-TES-85	UniProtKB: B2RBQ6	1917	1918	1919, 1920, 1921, 1922, 1923
HOM-TES-85	UniProtKB: Q9P127	1924	1925	1926, 1927, 1928, 1929, 1930
HPG1	Pubmed: 12543784	1931	1932	1933, 1934, 1935, 1936, 1937
HS71A	UniProtKB: P0DMV8	1938	1939	1940, 1941, 1942, 1943, 1944
HS71B	UniProtKB: P0DMV9	1945	1946	1947, 1948, 1949, 1950, 1951
HST-2	UniProtKB: P10767	1952	1953	1954, 1955, 1956, 1957, 1958
hTERT	UniProtKB: O94807	1959	1960	1961, 1962, 1963, 1964, 1965
iCE	UniProtKB: O00748	1966	1967	1968, 1969, 1970, 1971, 1972
IF2B3	UniProtKB: O00425	1973	1974	1975, 1976, 1977, 1978, 1979
IL-13Ra2	UniProtKB: Q14627	1980	1981	1982, 1983, 1984, 1985, 1986
IL2-RA	UniProtKB: P01589	1987	1988	1989, 1990, 1991, 1992, 1993
IL2-RB	UniProtKB: P14784	1994	1995	1996, 1997, 1998, 1999, 2000
IL2-RG	UniProtKB: P31785	2001	2002	2003, 2004, 2005, 2006, 2007
IMP3	UniProtKB: Q9NV31	2008	2009	2010, 2011, 2012,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				2013, 2014
ITA5	UniProtKB: P08648	2015	2016	2017, 2018, 2019, 2020, 2021
ITB1	UniProtKB: P05556	2022	2023	2024, 2025, 2026, 2027, 2028
ITB6	UniProtKB: P18564	2029	2030	2031, 2032, 2033, 2034, 2035
kallikrein-2	UniProtKB: A0A024R4J4	2036	2037	2038, 2039, 2040, 2041, 2042
kallikrein-2	UniProtKB: A0A024R4N3	2043	2044	2045, 2046, 2047, 2048, 2049
kallikrein-2	UniProtKB: B0AZU9	2050	2051	2052, 2053, 2054, 2055, 2056
kallikrein-2	UniProtKB: B4DU77	2057	2058	2059, 2060, 2061, 2062, 2063
kallikrein-2	UniProtKB: P20151	2064	2065	2066, 2067, 2068, 2069, 2070
kallikrein-2	UniProtKB: Q6T774	2071	2072	2073, 2074, 2075, 2076, 2077
kallikrein-2	UniProtKB: Q6T775	2078	2079	2080, 2081, 2082, 2083, 2084
kallikrein-4	UniProtKB: A0A0C4DFQ5	2085	2086	2087, 2088, 2089, 2090, 2091
kallikrein-4	UniProtKB: Q5BQA0	2092	2093	2094, 2095, 2096, 2097, 2098
kallikrein-4	UniProtKB: Q96PT0	2099	2100	2101, 2102, 2103, 2104, 2105
kallikrein-4	UniProtKB: Q96PT1	2106	2107	2108, 2109, 2110, 2111, 2112
kallikrein-4	UniProtKB: Q9Y5K2	2113	2114	2115, 2116, 2117, 2118, 2119
KI20A	UniProtKB: O95235	2120	2121	2122, 2123, 2124, 2125, 2126
KIAA0205	UniProtKB: Q92604	2127	2128	2129, 2130, 2131, 2132, 2133
KIF2C	UniProtKB: Q99661	2134	2135	2136, 2137, 2138, 2139, 2140
KK-LC-1	UniProtKB: Q5H943	2141	2142	2143, 2144, 2145, 2146, 2147
LDLR	UniProtKB: P01130	2148	2149	2150, 2151, 2152, 2153, 2154

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
LGMN	UniProtKB: Q99538	2155	2156	2157, 2158, 2159, 2160, 2161
LIRB2	UniProtKB: Q8N423	2162	2163	2164, 2165, 2166, 2167, 2168
LY6K	UniProtKB: Q17RY6	2169	2170	2171, 2172, 2173, 2174, 2175
MAGA5	UniProtKB: P43359	2176	2177	2178, 2179, 2180, 2181, 2182
MAGA8	UniProtKB: P43361	2183	2184	2185, 2186, 2187, 2188, 2189
MAGAB	UniProtKB: P43364	2190	2191	2192, 2193, 2194, 2195, 2196
MAGE-A10	UniProtKB: A0A024RC14	2197	2198	2199, 2200, 2201, 2202, 2203
MAGE-A12	UniProtKB: P43365	2204	2205	2206, 2207, 2208, 2209, 2210
MAGE-A1	UniProtKB: P43355	2211	2212	2213, 2214, 2215, 2216, 2217
MAGE-A2	UniProtKB: P43356	2218	2219	2220, 2221, 2222, 2223, 2224
MAGE-A3	UniProtKB: P43357	2225	2226	2227, 2228, 2229, 2230, 2231
MAGE-A4	UniProtKB: A0A024RC12	2232	2233	2234, 2235, 2236, 2237, 2238
MAGE-A4	UniProtKB: P43358	2239	2240	2241, 2242, 2243, 2244, 2245
MAGE-A4	UniProtKB: Q1RN33	2246	2247	2248, 2249, 2250, 2251, 2252
MAGE-A6	UniProtKB: A8K072	2253	2254	2255, 2256, 2257, 2258, 2259
MAGE-A6	UniProtKB: P43360	2260	2261	2262, 2263, 2264, 2265, 2266
MAGE-A6	UniProtKB: Q6FHI5	2267	2268	2269, 2270, 2271, 2272, 2273
MAGE-A9	UniProtKB: P43362	2274	2275	2276, 2277, 2278, 2279, 2280
MAGE-B10	UniProtKB: Q96LZ2	2281	2282	2283, 2284, 2285, 2286, 2287
MAGE-B16	UniProtKB: A2A368	2288	2289	2290, 2291, 2292, 2293, 2294
MAGE-B17	UniProtKB: A8MXT2	2295	2296	2297, 2298, 2299,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				2300, 2301
MAGE-_B1	UniProtKB: Q96TG1	2302	2303	2304, 2305, 2306, 2307, 2308
MAGE-B2	UniProtKB: O15479	2309	2310	2311, 2312, 2313, 2314, 2315
MAGE-B3	UniProtKB: O15480	2316	2317	2318, 2319, 2320, 2321, 2322
MAGE-B4	UniProtKB: O15481	2323	2324	2325, 2326, 2327, 2328, 2329
MAGE-B5	UniProtKB: Q9BZ81	2330	2331	2332, 2333, 2334, 2335, 2336
MAGE-B6	UniProtKB: Q8N7X4	2337	2338	2339, 2340, 2341, 2342, 2343
MAGE-C1	UniProtKB: O60732	2344	2345	2346, 2347, 2348, 2349, 2350
MAGE-C2	UniProtKB: Q9UBF1	2351	2352	2353, 2354, 2355, 2356, 2357
MAGE-C3	UniProtKB: Q8TD91	2358	2359	2360, 2361, 2362, 2363, 2364
MAGE-D1	UniProtKB: Q9Y5V3	2365	2366	2367, 2368, 2369, 2370, 2371
MAGE-D2	UniProtKB: Q9UNF1	2372	2373	2374, 2375, 2376, 2377, 2378
MAGE-D4	UniProtKB: Q96JG8	2379	2380	2381, 2382, 2383, 2384, 2385
MAGE-_E1	UniProtKB: Q6IAI7	2386	2387	2388, 2389, 2390, 2391, 2392
MAGE-E1_(MAGE1)	UniProtKB: Q9HCI5	2393	2394	2395, 2396, 2397, 2398, 2399
MAGE-E2	UniProtKB: Q8TD90	2400	2401	2402, 2403, 2404, 2405, 2406
MAGE-F1	UniProtKB: Q9HAY2	2407	2408	2409, 2410, 2411, 2412, 2413
MAGE-H1	UniProtKB: Q9H213	2414	2415	2416, 2417, 2418, 2419, 2420
MAGEL2	UniProtKB: Q9UJ55	2421	2422	2423, 2424, 2425, 2426, 2427
mammaglobin_A	UniProtKB: Q13296	2428	2429	2430, 2431, 2432, 2433, 2434
mammaglobin_A	UniProtKB: Q6NX70	2435	2436	2437, 2438, 2439, 2440, 2441

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
MART-1/melan-A	UniProtKB: Q16655	2442	2443	2444, 2445, 2446, 2447, 2448
MART-2	UniProtKB: Q5VTY9	2449	2450	2451, 2452, 2453, 2454, 2455
MC1_R	UniProtKB: Q01726	2456	2457	2458, 2459, 2460, 2461, 2462
MC1_R	UniProtKB: Q1JUL4	2463	2464	2465, 2466, 2467, 2468, 2469
MC1_R	UniProtKB: Q1JUL6	2470	2471	2472, 2473, 2474, 2475, 2476
MC1_R	UniProtKB: Q1JUL8	2477	2478	2479, 2480, 2481, 2482, 2483
MC1_R	UniProtKB: Q1JUL9	2484	2485	2486, 2487, 2488, 2489, 2490
MC1_R	UniProtKB: Q1JUM0	2491	2492	2493, 2494, 2495, 2496, 2497
MC1_R	UniProtKB: Q1JUM2	2498	2499	2500, 2501, 2502, 2503, 2504
MC1_R	UniProtKB: Q1JUM3	2505	2506	2507, 2508, 2509, 2510, 2511
MC1_R	UniProtKB: Q1JUM4	2512	2513	2514, 2515, 2516, 2517, 2518
MC1_R	UniProtKB: Q1JUM5	2519	2520	2521, 2522, 2523, 2524, 2525
MC1_R	UniProtKB: Q6UR92	2526	2527	2528, 2529, 2530, 2531, 2532
MC1_R	UniProtKB: Q6UR94	2533	2534	2535, 2536, 2537, 2538, 2539
MC1_R	UniProtKB: Q6UR95	2540	2541	2542, 2543, 2544, 2545, 2546
MC1_R	UniProtKB: Q6UR96	2547	2548	2549, 2550, 2551, 2552, 2553
MC1_R	UniProtKB: Q6UR97	2554	2555	2556, 2557, 2558, 2559, 2560
MC1_R	UniProtKB: Q6UR98	2561	2562	2563, 2564, 2565, 2566, 2567
MC1_R	UniProtKB: Q6UR99	2568	2569	2570, 2571, 2572, 2573, 2574
MC1_R	UniProtKB: Q6URA0	2575	2576	2577, 2578, 2579, 2580, 2581
MC1_R	UniProtKB: Q86YW1	2582	2583	2584, 2585, 2586,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				2587, 2588
MC1_R	UniProtKB: V9Q5S2	2589	2590	2591, 2592, 2593, 2594, 2595
MC1_R	UniProtKB: V9Q671	2596	2597	2598, 2599, 2600, 2601, 2602
MC1_R	UniProtKB: V9Q783	2603	2604	2605, 2606, 2607, 2608, 2609
MC1_R	UniProtKB: V9Q7F1	2610	2611	2612, 2613, 2614, 2615, 2616
MC1_R	UniProtKB: V9Q8N1	2617	2618	2619, 2620, 2621, 2622, 2623
MC1_R	UniProtKB: V9Q977	2624	2625	2626, 2627, 2628, 2629, 2630
MC1_R	UniProtKB: V9Q9P5	2631	2632	2633, 2634, 2635, 2636, 2637
MC1_R	UniProtKB: V9Q9R8	2638	2639	2640, 2641, 2642, 2643, 2644
MC1_R	UniProtKB: V9QAE0	2645	2646	2647, 2648, 2649, 2650, 2651
MC1_R	UniProtKB: V9QAR2	2652	2653	2654, 2655, 2656, 2657, 2658
MC1_R	UniProtKB: V9QAW3	2659	2660	2661, 2662, 2663, 2664, 2665
MC1_R	UniProtKB: V9QB02	2666	2667	2668, 2669, 2670, 2671, 2672
MC1_R	UniProtKB: V9QB58	2673	2674	2675, 2676, 2677, 2678, 2679
MC1_R	UniProtKB: V9QBY6	2680	2681	2682, 2683, 2684, 2685, 2686
MC1_R	UniProtKB: V9QC17	2687	2688	2689, 2690, 2691, 2692, 2693
MC1_R	UniProtKB: V9QC66	2694	2695	2696, 2697, 2698, 2699, 2700
MC1_R	UniProtKB: V9QCQ4	2701	2702	2703, 2704, 2705, 2706, 2707
MC1_R	UniProtKB: V9QDF4	2708	2709	2710, 2711, 2712, 2713, 2714
MC1_R	UniProtKB: V9QDN7	2715	2716	2717, 2718, 2719, 2720, 2721
MC1_R	UniProtKB: V9QDQ6	2722	2723	2724, 2725, 2726, 2727, 2728

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
mesothelin	UniProtKB: Q13421	2729	2730	2731, 2732, 2733, 2734, 2735
MITF	UniProtKB: O75030-8	2736	2737	2738, 2739, 2740, 2741, 2742
MITF	UniProtKB: O75030-9	2743	2744	2745, 2746, 2747, 2748, 2749
MITF	UniProtKB: O75030	2750	2751	2752, 2753, 2754, 2755, 2756
MMP1_1	UniProtKB: B3KQS8	2757	2758	2759, 2760, 2761, 2762, 2763
MMP7	UniProtKB: P09237	2764	2765	2766, 2767, 2768, 2769, 2770
MUC-1	Genbank: AAA60019	2771	2772	2773, 2774, 2775, 2776, 2777
MUM-1/m	RefSeq: NP_116242	2778	2779	2780, 2781, 2782, 2783, 2784
MUM-2/m	UniProtKB: Q9Y5R8	2785	2786	2787, 2788, 2789, 2790, 2791
MYO1A	UniProtKB: Q9UBC5	2792	2793	2794, 2795, 2796, 2797, 2798
MYO1B	UniProtKB: O43795	2799	2800	2801, 2802, 2803, 2804, 2805
MYO1C	UniProtKB: O00159	2806	2807	2808, 2809, 2810, 2811, 2812
MYO1D	UniProtKB: O94832	2813	2814	2815, 2816, 2817, 2818, 2819
MYO1E	UniProtKB: Q12965	2820	2821	2822, 2823, 2824, 2825, 2826
MYO1F	UniProtKB: O00160	2827	2828	2829, 2830, 2831, 2832, 2833
MYO1G	UniProtKB: B0I1T2	2834	2835	2836, 2837, 2838, 2839, 2840
MYO1H	RefSeq: NP_001094891	2841	2842	2843, 2844, 2845, 2846, 2847
NA17	UniProtKB: Q3V5L5	2848	2849	2850, 2851, 2852, 2853, 2854
NA88-A	Pubmed: 10790436	2855	2856	2857, 2858, 2859, 2860, 2861
Neo-PAP	UniProtKB: Q9BWT3	2862	2863	2864, 2865, 2866, 2867, 2868
NFYC/m	UniProtKB: Q13952	2869	2870	2871, 2872, 2873,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				2874, 2875
NGEP	UniProtKB: Q6IWH7	2876	2877	2878, 2879, 2880, 2881, 2882
NPM	UniProtKB: P06748	2883	2884	2885, 2886, 2887, 2888, 2889
NRCAM	UniProtKB: Q92823	2890	2891	2892, 2893, 2894, 2895, 2896
NSE	UniProtKB: P09104	2897	2898	2899, 2900, 2901, 2902, 2903
NUF2	UniProtKB: Q9BZD4	2904	2905	2906, 2907, 2908, 2909, 2910
NY-ESO-1	UniProtKB: P78358	2911	2912	2913, 2914, 2915, 2916, 2917
OA1	UniProtKB: P51810	2918	2919	2920, 2921, 2922, 2923, 2924
OGT	UniProtKB: O15294	2925	2926	2927, 2928, 2929, 2930, 2931
OS-9	UniProtKB: B4DH11	2932	2933	2934, 2935, 2936, 2937, 2938
OS-9	UniProtKB: B4E321	2939	2940	2941, 2942, 2943, 2944, 2945
OS-9	UniProtKB: B7Z8E7	2946	2947	2948, 2949, 2950, 2951, 2952
OS-9	UniProtKB: Q13438	2953	2954	2955, 2956, 2957, 2958, 2959
osteocalcin	UniProtKB: P02818	2960	2961	2962, 2963, 2964, 2965, 2966
osteopontin	UniProtKB: A0A024RDE2	2967	2968	2969, 2970, 2971, 2972, 2973
osteopontin	UniProtKB: A0A024RDE6	2974	2975	2976, 2977, 2978, 2979, 2980
osteopontin	UniProtKB: A0A024RDJ0	2981	2982	2983, 2984, 2985, 2986, 2987
osteopontin	UniProtKB: B7Z351	2988	2989	2990, 2991, 2992, 2993, 2994
osteopontin	UniProtKB: F2YQ21	2995	2996	2997, 2998, 2999, 3000, 3001
osteopontin	UniProtKB: P10451	3002	3003	3004, 3005, 3006, 3007, 3008
p53	UniProtKB: P04637	3009	3010	3011, 3012, 3013, 3014, 3015

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
PAGE-4	UniProtKB: O60829	3016	3017	3018, 3019, 3020, 3021, 3022
PAI-1	UniProtKB: P05121	3023	3024	3025, 3026, 3027, 3028, 3029
PAI-2	UniProtKB: P05120	3030	3031	3032, 3033, 3034, 3035, 3036
PAP	UniProtKB: Q06141	3037	3038	3039, 3040, 3041, 3042, 3043
PAP	UniProtKB: Q53S56	3044	3045	3046, 3047, 3048, 3049, 3050
PATE	UniProtKB: Q8WXA2	3051	3052	3053, 3054, 3055, 3056, 3057
PAX3	UniProtKB: P23760	3058	3059	3060, 3061, 3062, 3063, 3064
PAX5	UniProtKB: Q02548	3065	3066	3067, 3068, 3069, 3070, 3071
PD1L1	UniProtKB: Q9NZQ7	3072	3073	3074, 3075, 3076, 3077, 3078
PDCD1	UniProtKB: Q15116	3079	3080	3081, 3082, 3083, 3084, 3085
PDEF	UniProtKB: O95238	3086	3087	3088, 3089, 3090, 3091, 3092
PECA1	UniProtKB: P16284	3093	3094	3095, 3096, 3097, 3098, 3099
PGCB	UniProtKB: Q96GW7	3100	3101	3102, 3103, 3104, 3105, 3106
PGFRB	UniProtKB: P09619	3107	3108	3109, 3110, 3111, 3112, 3113
Pim-1_-Kinase	UniProtKB: A0A024RD25	3114	3115	3116, 3117, 3118, 3119, 3120
Pin-1	UniProtKB: O15428	3121	3122	3123, 3124, 3125, 3126, 3127
Pin-1	UniProtKB: Q13526	3128	3129	3130, 3131, 3132, 3133, 3134
Pin-1	UniProtKB: Q49AR7	3135	3136	3137, 3138, 3139, 3140, 3141
PLAC1	UniProtKB: Q9HBJ0	3142	3143	3144, 3145, 3146, 3147, 3148
PMEL	UniProtKB: P40967	3149	3150	3151, 3152, 3153, 3154, 3155
PML	UniProtKB: P29590	3156	3157	3158, 3159, 3160,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				3161, 3162
POTEF	UniProtKB: A5A3E0	3163	3164	3165, 3166, 3167, 3168, 3169
POTE	UniProtKB: Q86YR6	3170	3171	3172, 3173, 3174, 3175, 3176
PRAME	UniProtKB: A0A024R1E6	3177	3178	3179, 3180, 3181, 3182, 3183
PRAME	UniProtKB: P78395	3184	3185	3186, 3187, 3188, 3189, 3190
PRDX5/m	UniProtKB: P30044	3191	3192	3193, 3194, 3195, 3196, 3197
PRM2	UniProtKB: P04554	3198	3199	3200, 3201, 3202, 3203, 3204
prostein	UniProtKB: Q96JT2	3205	3206	3207, 3208, 3209, 3210, 3211
proteinase-3	UniProtKB: D6CHE9	3212	3213	3214, 3215, 3216, 3217, 3218
proteinase-3	UniProtKB: P24158	3219	3220	3221, 3222, 3223, 3224, 3225
PSA	UniProtKB: P55786	3226	3227	3228, 3229, 3230, 3231, 3232
PSB9	UniProtKB: P28065	3233	3234	3235, 3236, 3237, 3238, 3239
PSCA	UniProtKB: D3DWI6	3240	3241	3242, 3243, 3244, 3245, 3246
PSCA	UniProtKB: O43653	3247	3248	3249, 3250, 3251, 3252, 3253
PSGR	UniProtKB: Q9H255	3254	3255	3256, 3257, 3258, 3259, 3260
PSM	UniProtKB: Q04609	3261	3262	3263, 3264, 3265, 3266, 3267
PTPRC	RefSeq: NP_002829	3268	3269	3270, 3271, 3272, 3273, 3274
RAB8A	UniProtKB: P61006	3275	3276	3277, 3278, 3279, 3280, 3281
RAGE-1	UniProtKB: Q9UQ07	3282	3283	3284, 3285, 3286, 3287, 3288
RARA	UniProtKB: P10276	3289	3290	3291, 3292, 3293, 3294, 3295
RASH	UniProtKB: P01112	3296	3297	3298, 3299, 3300, 3301, 3302

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
RASK	UniProtKB: P01116	3303	3304	3305, 3306, 3307, 3308, 3309
RASN	UniProtKB: P01111	3310	3311	3312, 3313, 3314, 3315, 3316
RGS5	UniProtKB: O15539	3317	3318	3319, 3320, 3321, 3322, 3323
RHAMM/CD168	UniProtKB: O75330	3324	3325	3326, 3327, 3328, 3329, 3330
RHOC	UniProtKB: P08134	3331	3332	3333, 3334, 3335, 3336, 3337
RSSA	UniProtKB: P08865	3338	3339	3340, 3341, 3342, 3343, 3344
RU1	UniProtKB: Q9UHJ3	3345	3346	3347, 3348, 3349, 3350, 3351
RU2	UniProtKB: Q9UHG0	3352	3353	3354, 3355, 3356, 3357, 3358
RUNX1	UniProtKB: Q01196	3359	3360	3361, 3362, 3363, 3364, 3365
S-100	UniProtKB: V9HW39	3366	3367	3368, 3369, 3370, 3371, 3372
SAGE	UniProtKB: Q9NXZ1	3373	3374	3375, 3376, 3377, 3378, 3379
SART-_1	UniProtKB: O43290	3380	3381	3382, 3383, 3384, 3385, 3386
SART-2	UniProtKB: Q9UL01	3387	3388	3389, 3390, 3391, 3392, 3393
SART-3	UniProtKB: Q15020	3394	3395	3396, 3397, 3398, 3399, 3400
SEPR	UniProtKB: Q12884	3401	3402	3403, 3404, 3405, 3406, 3407
SIA7F	UniProtKB: Q969X2	3408	3409	3410, 3411, 3412, 3413, 3414
SIA8A	UniProtKB: Q92185	3415	3416	3417, 3418, 3419, 3420, 3421
SIAT9	UniProtKB: Q9UNP4	3422	3423	3424, 3425, 3426, 3427, 3428
SIRT2/m	UniProtKB: A0A024R0G8	3429	3430	3431, 3432, 3433, 3434, 3435
SIRT2/m	UniProtKB: Q8IXJ6	3436	3437	3438, 3439, 3440, 3441, 3442
SOX10	UniProtKB: P56693	3443	3444	3445, 3446, 3447,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				3448, 3449
SP17	UniProtKB: Q15506	3450	3451	3452, 3453, 3454, 3455, 3456
SPNXA	UniProtKB: Q9NS26	3457	3458	3459, 3460, 3461, 3462, 3463
SPXN3	UniProtKB: Q5MJ09	3464	3465	3466, 3467, 3468, 3469, 3470
SSX-1	UniProtKB: Q16384	3471	3472	3473, 3474, 3475, 3476, 3477
SSX-2	UniProtKB: Q16385	3478	3479	3480, 3481, 3482, 3483, 3484
SSX3	UniProtKB: Q99909	3485	3486	3487, 3488, 3489, 3490, 3491
SSX-4	UniProtKB: O60224	3492	3493	3494, 3495, 3496, 3497, 3498
ST1A1	UniProtKB: P50225	3499	3500	3501, 3502, 3503, 3504, 3505
STAG2	UniProtKB: Q8N3U4-2	3506	3507	3508, 3509, 3510, 3511, 3512
STAMP-1	UniProtKB: Q8NFT2	3513	3514	3515, 3516, 3517, 3518, 3519
STEAP-1	UniProtKB: A0A024RA63	3520	3521	3522, 3523, 3524, 3525, 3526
STEAP-1	UniProtKB: Q9UHE8	3527	3528	3529, 3530, 3531, 3532, 3533
Survivin-2B	UniProtKB: O15392-2	3534	3535	3536, 3537, 3538, 3539, 3540
survivin	UniProtKB: O15392	3541	3542	3543, 3544, 3545, 3546, 3547
SYCP1	UniProtKB: A0A024R0I2	3548	3549	3550, 3551, 3552, 3553, 3554
SYCP1	UniProtKB: B7ZLS9	3555	3556	3557, 3558, 3559, 3560, 3561
SYCP1	UniProtKB: Q15431	3562	3563	3564, 3565, 3566, 3567, 3568
SYCP1	UniProtKB: Q3MHC4	3569	3570	3571, 3572, 3573, 3574, 3575
SYT-SSX-1	UniProtKB: A4PIV7	3576	3577	3578, 3579, 3580, 3581, 3582
SYT-SSX-1	UniProtKB: A4PIV8	3583	3584	3585, 3586, 3587, 3588, 3589

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
SYT-SSX-2	UniProtKB: A4PIV9	3590	3591	3592, 3593, 3594, 3595, 3596
SYT-SSX-2	UniProtKB: A4PIW0	3597	3598	3599, 3600, 3601, 3602, 3603
TARP	UniProtKB: Q0VGM3	3604	3605	3606, 3607, 3608, 3609, 3610
TCRg	UniProtKB: A2JGV3	3611	3612	3613, 3614, 3615, 3616, 3617
TF2AA	UniProtKB: P52655	3618	3619	3620, 3621, 3622, 3623, 3624
TGFR2	UniProtKB: P37173	3625	3626	3627, 3628, 3629, 3630, 3631
TGM-4	UniProtKB: B2R7D1	3632	3633	3634, 3635, 3636, 3637, 3638
TIE2	UniProtKB: Q02763	3639	3640	3641, 3642, 3643, 3644, 3645
TKTL1	UniProtKB: P51854	3646	3647	3648, 3649, 3650, 3651, 3652
TPI/m	UniProtKB: P60174	3653	3654	3655, 3656, 3657, 3658, 3659
TRGV11	UniProtKB: Q99601	3660	3661	3662, 3663, 3664, 3665, 3666
TRGV9	UniProtKB: A4D1X2	3667	3668	3669, 3670, 3671, 3672, 3673
TRGV9	UniProtKB: Q99603	3674	3675	3676, 3677, 3678, 3679, 3680
TRGV9	UniProtKB: Q99604	3681	3682	3683, 3684, 3685, 3686, 3687
TRPC1	UniProtKB: P48995	3688	3689	3690, 3691, 3692, 3693, 3694
TRP-p8	UniProtKB: Q7Z2W7	3695	3696	3697, 3698, 3699, 3700, 3701
TSG10	UniProtKB: Q9BZW7	3702	3703	3704, 3705, 3706, 3707, 3708
TSPY1	UniProtKB: Q01534	3709	3710	3711, 3712, 3713, 3714, 3715
TVC_(TRGV3)	Genbank: M13231.1	3716	3717	3718, 3719, 3720, 3721, 3722
TX101	UniProtKB: Q9BY14-2	3723	3724	3725, 3726, 3727, 3728, 3729
tyrosinase	UniProtKB: A0A024DBG7	3730	3731	3732, 3733, 3734,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
				3735, 3736
tyrosinase	UniProtKB: L8B082	3737	3738	3739, 3740, 3741, 3742, 3743
tyrosinase	UniProtKB: L8B086	3744	3745	3746, 3747, 3748, 3749, 3750
tyrosinase	UniProtKB: L8B0B9	3751	3752	3753, 3754, 3755, 3756, 3757
tyrosinase	UniProtKB: O75767	3758	3759	3760, 3761, 3762, 3763, 3764
tyrosinase	UniProtKB: P14679	3765	3766	3767, 3768, 3769, 3770, 3771
tyrosinase	UniProtKB: U3M8N0	3772	3773	3774, 3775, 3776, 3777, 3778
tyrosinase	UniProtKB: U3M9D5	3779	3780	3781, 3782, 3783, 3784, 3785
tyrosinase	UniProtKB: U3M9J2	3786	3787	3788, 3789, 3790, 3791, 3792
TYRP1	UniProtKB: P17643	3793	3794	3795, 3796, 3797, 3798, 3799
TYRP2	UniProtKB: P40126	3800	3801	3802, 3803, 3804, 3805, 3806
UPA	UniProtKB: Q96NZ9	3807	3808	3809, 3810, 3811, 3812, 3813
VEGFR1	UniProtKB: B5A924	3814	3815	3816, 3817, 3818, 3819, 3820
WT1	UniProtKB: A0A0H5AUY0	3821	3822	3823, 3824, 3825, 3826, 3827
WT1	UniProtKB: P19544	3828	3829	3830, 3831, 3832, 3833, 3834
WT1	UniProtKB: Q06250	3835	3836	3837, 3838, 3839, 3840, 3841
XAGE1	UniProtKB: Q9HD64	3842	3843	3844, 3845, 3846, 3847, 3848
IL-10	UniProtKB: P22301	4169	4170	4171, 4172, 4173, 4174, 4175, 4176
IL-5	UniProtKB: P05113	4585	4586	4587, 4588, 4589, 4590, 4591, 4592
M-CSF	UniProtKB: P09603	4705	4706	4707, 4708, 4709, 4710, 4711, 4712
TGFbeta1	UniProtKB: P01137	4785	4786	4787, 4788, 4789, 4790, 4791, 4792

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
Caspase_8	UniProtKB: Q14790	7113	7114	7115, 7116, 7117, 7118, 7119, 7120
SERPINB5	UniProtKB: P36952	7465	7466	7467, 7468, 7469, 7470, 7471, 7472
calreticulin	UniProtKB: B4DHR1	7569	7570	7571, 7572, 7573, 7574, 7575, 7576
calreticulin	UniProtKB: B4E2Y9	7577	7578	7579, 7580, 7581, 7582, 7583, 7584
calreticulin	UniProtKB: P27797	7585	7586	7587, 7588, 7589, 7590, 7591, 7592
calreticulin	UniProtKB: Q96L12	7593	7594	7595, 7596, 7597, 7598, 7599, 7600
N-myc	UniProtKB: P04198	9987	9988	9989, 9990, 9991, 9992, 9993, 9994

According to the present invention, in a more preferred embodiment, the inventive composition comprises at least one RNA, preferably an mRNA comprising at least one coding region encoding at least one tumor antigen or a fragment or variant thereof, wherein the at least one coding region comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequences according to the SEQ ID Nos as disclosed in Table 9.

Furthermore tumor antigens also may encompass idiotypic antigens associated with a cancer or tumor disease, particularly lymphoma or a lymphoma associated disease, wherein said idiotypic antigen is an immunoglobulin idiotype of a lymphoid blood cell or a T cell receptor idiotype of a lymphoid blood cell.

In a particularly preferred embodiment the inventive RNA composition comprises at least one RNA, wherein the at least one RNA encodes the following antigens:

- STEAP (Six Transmembrane Epithelial Antigen of the Prostate);
- PSA (Prostate-Specific Antigen),
- PSMA (Prostate-Specific Membrane Antigen),

- PSCA (Prostate Stem Cell Antigen);
- PAP (Prostatic Acid Phosphatase), and
- MUC1 (Mucin 1).

In another particularly preferred embodiment the inventive RNA composition comprises at least one RNA, wherein the at least one RNA encodes the following antigens:

- 5T4 (Trophoblast glycoprotein, TPBG);
- Survivin (Baculoviral IAP repeat-containing protein 5; BIRC5),
- NY-ESO-1 (New York esophageal squamous cell carcinoma 1; CTAG1B),
- MAGE-C1 (Melanoma antigen family C1);
- MAGE-C2 (Melanoma antigen family C2), and
- MUC1 (Mucin 1).

9. β-catenin inhibitors

In a further preferred embodiment of the inventive RNA containing composition the RNA, preferably mRNA codes for at least one β-catenin inhibitor or a fragment or variant thereof. Preferably the RNA encoding the at least one β-catenin inhibitor encodes an inhibitory protein or dominant negative mutant protein of the β-catenin pathway. Particular preferred β-catenin inhibitors according to the present invention comprise TAT-NLS-BLBD-6, axin-1, TCF-4, GSK-3b, DKK-1, Dvl-1 derivatives or fragments thereof.

As reviewed by Thakur and Mishra (Thakur R, Mishra DP. Pharmacological modulation of beta-catenin and its applications in cancer therapy. J Cell Mol Med. 2013 Apr;17(4):449-56. doi: 10.1111/jcmm.12033) beta-catenin (β-catenin) is a multifunctional protein which plays an important role in physiological homeostasis. It acts both as a transcriptional regulator and an adaptor protein for intracellular adhesion. β-catenin is necessary for the establishment and maintance of epithelial layers and provides a linkage between intracellular junctions and cytoskeletal proteins. β-catenin is regulated by Wnt signaling. In the absence of Wnt downstream signal β-catenin is phosphorylated which leads to its ubiquitination and eventually protein degradation. Various literature reports have linked β-catenin to the malignant transformation of normal cells. For example, Wnt signaling and β-catenin nuclear localization was associated with

differentiation of hepatocytes into a tumoral phenotype. Similarly, in lung epithelial and pancreatic cells, activation of β-catenin was sufficient for induction of oncogenic transformation. In addition to being a driving force of malignant transformation, abnormal β-catenin expression and localization has been associated with increased metastatic potential. Recently, it has been shown that β-catenin signaling prevents T cell infiltration and anti-tumor immunity strongly limiting the potential effects of immunotherapies. Since β-catenin plays an important and detrimental role in tumorigenesis, it has been proposed as a putative drug target.

10. STING-pathway activators

In a further preferred embodiment of the inventive RNA containing composition the RNA, preferably mRNA codes for at least one activator of the STING (stimulator of interferon genes) pathway or a fragment or variant thereof. Preferably, the RNA encoding the at least one activator (stimulator) of the STING pathway encodes an activating protein or a constitutively active protein of the STING pathway, preferably DDX41, STING, cGAS, IRF3, TBK1 or STAT6 or a fragment or variant thereof.

As reviewed by Woo et al. (Woo SR, Corrales L, Gajewski TF. The STING pathway and the T cell-inflamed tumor microenvironment. Trends Immunol. 2015 Mar 7. pii: S1471-4906(15)00019-8. doi: 10.1016/j.it.2015.02.003) and Dubensky et al. (Dubensky TW Jr, Kanne DB, Leong ML. Rationale, progress and development of vaccines utilizing STING-activating cyclic dinucleotide adjuvants. Ther Adv Vaccines. 2013 Nov;1(4):131-43. doi: 10.1177/2051013613501988) the so-called STING pathway (STING - stimulator of interferon genes) is responsible for sensing of cytoplasmic DNA and induction of proinflammatory mediators. After binding of DNA in cytoplasm, STING activates signaling via TANK-binding kinase 1 (TBK-1)/IRF-3 axis which results in production of IFN-β. This pathway was shown to play an important role in sensing of DNA viruses as well as some autoimmune disorders. Recent data have identified STING pathway as absolutely necessary to induce spontaneous T cell priming against tumor antigens *in vivo*. Tumor DNA was detected within tumor-infiltrating DCs, which led to IFN-β production and T cell activation. Thus, intratumoral application of small molecules STING pathway agonists has demonstrated their efficacy in tumor-bearing animals. Agonists of the STING pathway has been also evaluated as vaccine adjuvants showing potency to induce cellular and humoral immunity in vaccinated hosts.

11. Checkpoint modulators

In a further preferred embodiment of the inventive RNA containing composition the RNA, preferably mRNA comprises at least one coding region that codes for at least one checkpoint modulator or a fragment or variant thereof.

Negative regulatory T cell surface molecules were discovered which are upregulated in activated T cells to dampen their activity, resulting in less effective killing of tumor cells. These inhibitory molecules were termed negative co-stimulatory molecules due to their homology to the T cell co-stimulatory molecule CD28. These proteins, also referred to as immune checkpoint proteins, function in multiple pathways including the attenuation of early activation signals, competition for positive co-stimulation and direct inhibition of antigen presenting cells (Bour-Jordan et al., 2011. Immunol Rev. 241(1):180-205).

In preferred embodiments of the present invention the checkpoint modulator is a modulator of B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-H2, B7-H3, B7-H4, B7-H6, B7-H7/HHLA2, BTLA, CD28, CD28H/IGPR-1, CTLA-4, ICOS, PD-1, PD-L2/B7-DC, PDCD6, VISTA/B7-H5/PD-1H, BTN1A1/Butyrophilin, BTN2A1, BTN2A2/Butyrophilin 2A2, BTN3A1/2, BTN3A2, BTN3A3, BTNL2/Butyrophilin-like 2, BTNL3, BTNL4, BTNL6, BTNL8, BTNL9, BTNL10, CD277/BTN3A1, LAIR1, LAIR2, CD96, CD155/PVR, CRTAM, DNAM-1/CD226, Nectin-2/CD112, Nectin-3, TIGIT, LILRA3/CD85e, LILRA4/CD85g/ILT7, LILRB1/CD85j/ILT2, LILRB2/CD85d/ILT4, LILRB3/CD85a/ILT5, LILRB4/CD85k/ILT3, 4-1BB/TNFRSF9/CD137, 4-1BB Ligand/TNFSF9, BAFF/BLyS/TNFSF13B, BAFF R/TNFRSF13C, CD27/TNFRSF7, CD27 Ligand/TNFSF7, CD30/TNFRSF8, CD30 Ligand/TNFSF8, CD40/TNFRSF5, CD40 Ligand/TNFSF5, DR3/TNFRSF25, GITR/TNFRSF18, GITR Ligand/TNFSF18, HVEM/TNFRSF14, LIGHT/TNFSF14, Lymphotoxin-alpha/TNF-beta, OX40/TNFRSF4, OX40 Ligand/TNFSF4, RELT/TNFRSF19L, TACI/TNFRSF13B, TL1A/TNFSF15, TNF-alpha, TNF RII/TNFRSF1B, 2B4/CD244/SLAMF4, BLAME/SLAMF8, CD2, CD2F-10/SLAMF9, CD48/SLAMF2, CD58/LFA-3, CD84/SLAMF5, CD229/SLAMF3, CRACC/SLAMF7, NTB-A/SLAMF6, SLAM/CD150, TIM-1/KIM-1/HAVCR, TIM-3, TIM-4, CD7, CD96, CD160, CD200, CD300a/LMIR1, CRTAM, DAP12, Dectin-1/CLEC7A, DPPIV/CD26, EphB6, Integrin alpha 4 beta 1, Integrin alpha 4 beta 7/LPAM-1, LAG-3, TIM-1/KIM-1/HAVCR, TIM-4, TSLP R, or any combinations thereof.

In the context of the present invention a checkpoint modulator is defined herein as a molecule preferably a protein e.g. an antibody, a dominant negative receptor, a decoy receptor, or a ligand or a fragment or variant thereof, which modulates the function of an immune checkpoint protein, e.g. it inhibits or reduces the activity of checkpoint inhibitors (or inhibitory checkpoint molecules) or it stimulates the activity of checkpoint stimulators (or stimulatory checkpoint molecules). Therefore checkpoint modulators as defined herein, influence the activity of checkpoint molecules.

In this context inhibitory checkpoint molecules are defined as checkpoint inhibitors and can be used synonymously. In addition stimulatory checkpoint molecules are defined as checkpoint stimulators and can be used synonymously.

Preferable inhibitory checkpoint molecules that may be inhibited by a checkpoint modulator in the context of the invention are PD-1, PD-L1, CTLA-4, PD-L2, LAG3, TIM3/HAVCR2, 2B4, A2aR, B7H3, B7H4, BTLA, CD30, CD160, GAL9, HVEM, IDO1, IDO2, KIR, LAIR1 and VISTA.

Preferable stimulatory checkpoint molecules that may be stimulated by a checkpoint modulator in the context of the invention are CD2, CD27, CD28, CD40, CD137, CD226, CD276, GITR, ICOS, OX-40 and CD70.

Preferably, the checkpoint modulator is selected from agonistic antibodies, antagonistic antibodies, ligands, dominant negative receptors, and decoy receptors or combinations thereof.

Methods for generating and using mRNA-encoded antibodies are known in the art (e.g. WO2008/083949).

Preferably, the agonistic antibody is chosen from the following list: anti-4-1BB, anti-OX40, anti-GITR, anti-CD28, anti-CD27, anti-CD-40anti-ICOS, anti-TNFRSF25, and anti-LIGHT.

OX40 is a member of the TNFR-superfamily of receptors, and is expressed on the surface of antigen-activated mammalian CD4+ and CD8+ T lymphocytes. OX40 ligand (OX40L, also known as gp34, ACT-4-L, and CD252) is a protein that specifically interacts with the OX40 receptor. The term OX40L includes the entire OX40 ligand, soluble OX40 ligand, and fusion proteins comprising a

functionally active portion of OX40 ligand covalently linked to a second moiety, e.g., a protein domain. Also included within the definition of OX40L are variants which vary in amino acid sequence from naturally occurring OX4L but which retain the ability to specifically bind to the OX40 receptor. Further included within the definition of OX40L are variants which enhance the biological activity of OX40. An OX40 agonist is a molecule which induces or enhances the biological activity of OX40, e.g. signal transduction mediated by OX40. An OX40 agonist is preferably defined herein as a binding molecule capable of specific binding to OX40. Therefore, the OX40 agonist may be any agonist binding to OX40 and capable of stimulating OX40 signaling. In this context, the OX40 agonist may be an agonistic antibody binding to OX40.

OX40 agonists and anti-OX40 monoclonal antibodies are described in WO1995/021251, WO1995/012673 and WO1995/21915. Particularly preferred is the anti-OX40 antibody 9B12, a murine anti-OX40 monoclonal antibody directed against the extracellular domain of human OX40 (Weinberg et al., 2006. J. Immunother. 29(6):575-585).

Preferably, the antagonistic antibody is chosen from the list of anti-CTLA4, anti-PD1, anti-PD-L1, anti-Vista, anti-Tim-3, anti-LAG-3, and anti-BTLA.

Cytotoxic T lymphocyte antigen-4 (CTLA-4) is mainly expressed within the intracellular compartment of T cells. After a potent or long-lasting stimulus to a naive T cell via the T cell receptor (TCR), CTLA-4 is transported to the cell surface and concentrated at the immunological synapse. CTLA-4 then competes with CD28 for CD80/CD86 and down-modulates TCR signaling via effects on Akt signaling. Thus CTLA-4 functions physiologically as a signal dampener (Weber, J. 2010. Semin. Oncol. 37(5):430-9).

Particularly preferred are the anti-CTLA-4 antibodies ipilimumab (Yervoy®), tremelimumab, and AGEN-1884.

Members of the PD-1 pathway are all proteins which are associated with PD-1 signaling. On the one hand these might be proteins which induce PD-1 signaling upstream of PD-1 as e.g. the ligands of PD-1 PD-L1 and PD-L2 and the signal transduction receptor PD-1. On the other hand these might be signal transduction proteins downstream of PD-1 receptor. Particularly preferred as members of the PD-1 pathway in the context of the present invention are PD-1, PD-L1 and PD-L2.

In the context of the present invention, a PD-1 pathway antagonist is preferably defined herein as a compound capable to impair the PD-1 pathway signaling, preferably signaling mediated by the PD-1 receptor. Therefore, the PD-1 pathway antagonist may be any antagonist directed against any member of the PD-1 pathway capable of antagonizing PD-1 pathway signaling. In this context, the antagonist may be an antagonistic antibody as defined herein, targeting any member of the PD-1 pathway, preferably directed against PD-1 receptor, PD-L1 or PD-L2. This antagonistic antibody may also be encoded by a nucleic acid. Also, the PD-1 pathway antagonist may be a fragment of the PD-1 receptor blocking the activity of PD1 ligands. B7-1 or fragments thereof may act as PD1-antagonizing ligands as well. Additionally, a PD-1 pathway antagonist may be a protein comprising (or a nucleic acid coding for) an amino acid sequence capable of binding to PD-1 but preventing PD-1 signaling, e.g. by inhibiting PD-1 and B7-H1 or B7-DL interaction (WO2014127917).

Particularly preferred are the anti-PD1 antibodies Nivolumab (MDX-1106/BMS-936558/ONO-4538), (Brahmer et al., 2010. J Clin Oncol. 28(19):3167-75; PMID: 20516446); Pidilizumab (CT-011), (Berger et al., 2008. Clin Cancer Res. 14(10):3044-51; PMID: 18483370); Pembrolizumab (MK-3475, SCH 900475); AMP-224, and MEDI0680 (AMP-514)

Particularly preferred are the anti-PD-L1 antibodies MDX-1105/BMS-936559 (Brahmer et al. 2012. N Engl J Med. 366(26):2455-65; PMID: 22658128); atezolizumab (MPDL3280A/RG7446); durvalumab (MEDI4736); and avelumab (MSB0010718).

According to the present invention the at least one RNA of the inventive RNA containing composition encodes at least one antibody or fragments or variants thereof of Table 10. It is particularly preferred that the RNA containing composition comprises at least one RNA encoding the heavy chain of a particular antibody or fragments or variants thereof and at least one further RNA encoding the light chain of the same particular antibody or fragments or variants thereof.

Table 10: Antibodies directed against checkpoint molecules

Name	Target
Urelumab	4-1BB/CD137
PF-05082566	4-1BB/CD137
8H9	B7-H3

Enoblituzumab	B7-H3
Ipilimumab	CD152/CTLA-4
Ticilimumab (= tremelimumab)	CD152/CTLA-4
Tremelimumab	CD152/CTLA-4
Varlilumab	CD27
Teneliximab	CD40
Vorsetuzumab mafodotin	CD70
Lirilumab	KIR2D
GSK-3174998	OX40
MEDI-6469	OX40
MEDI-6383	OX40
MEDI-0562	OX40
PF-04518600	OX40
RG-7888	OX40
PF-06801591	PD-1
BGBA-317	PD-1
MEDI-0680	PD-1
MK-3475	PD-1
Nivolumab	PD-1
PDR-001	PD-1
Pembrolizumab	PD-1
Pidilizumab	PD-1
REGN-2810	PD-1
SHR-1210	PD-1
TSR-042	PD-1
MDX-1106	PD-1
Merck 3745	PD-1
CT- 011	PD-1
MEDI-0680	PD-1
PDR001	PD-1

REGN2810	PD-1
BGB-108	PD-1
BGB-A317	PD-1
AMP-224	PD-1
Atezolizumab	PD-L1 (CD274)
Avelumab	PD-L1 (CD274)
BMS-936559	PD-L1 (CD274)
Durvalumab	PD-L1 (CD274)
MEDI-4736	PD-L1 (CD274)
MPDL33280A	PD-L1 (CD274)
YW243.55.S70	PD-L1 (CD274)
MDX-1105	PD-L1 (CD274)
MSB0010718C	PD-L1 (CD274)

In a further preferred embodiment the checkpoint modulator is a decoy receptor (e.g. a soluble receptor). Preferably, the decoy receptor is a soluble PD1 receptor. In a particularly preferred embodiment the at least one RNA of the inventive RNA containing composition comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequence according to SEQ ID NO: 389 encoding a soluble PD-1 recptor.

In a further preferred embodiment of the inventive RNA containing composition the RNA, preferably an mRNA codes for at least one ligand which functions as a checkpoint modulator. Preferably, the ligand is CD40 Ligand (CD40L). In a further preferred embodiment of the inventive RNA containing composition the RNA, preferably an mRNA codes for at least one ligand which functions as a checkpoint modulator. Preferably, the ligand is CD40 Ligand (CD40L). Most preferably the at least one RNA of the inventive RNA containing composition comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequence according to SEQ ID NO: 10073 encoding CD40L.

12. Innate immune activators:

In this context innate immune activators may be selected from mammalian, in particular human adjuvant proteins, which typically comprise any human protein or peptide, 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 adjuvant proteins are selected from the group consisting of proteins which are components and ligands of the signalling networks of the pattern recognition receptors including TLR, NLR and RLH, including TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11; NOD1, NOD2, NOD3, NOD4, NOD5, NALP1, NALP2, NALP3, NALP4, NALP5, NALP6, NALP6, NALP7, NALP7, NALP8, NALP9, NALP10, NALP11, NALP12, NALP13, NALP14,I IPAF, NAIP, CIITA, RIG-I, MDA5 and LGP2, the signal transducers of TLR signaling including adaptor proteins including e.g. Trif and Cardif; components of the Small-GTPases signalling (RhoA, Ras, Rac1, Cdc42, Rab etc.), components of the PIP signalling (PI3K, Src-Kinases, etc.), components of the MyD88-dependent signalling (MyD88, IRAK1, IRAK2, IRAK4, TIRAP, TRAF6 etc.), components of the MyD88-independent signalling (TICAM1, TICAM2, TRAF6, TBK1, IRF3, TAK1, IRAK1 etc.); the activated kinases including e.g. Akt, MEKK1, MKK1, MKK3, MKK4, MKK6, MKK7, ERK1, ERK2, GSK3, PKC kinases, PKD kinases, GSK3 kinases, JNK, p38MAPK, TAK1, IKK, and TAK1; the activated transcription factors including e.g. NF-κB, c-Fos, c-Jun, c-Myc, CREB, AP-1, Elk-1, ATF2, IRF-3, IRF-7. Mammalian, in particular human adjuvant proteins may furthermore be selected from the group consisting of heat shock proteins, such as HSP10, HSP60, HSP65, HSP70, HSP75 and HSP90, gp96, Fibrinogen, TypIII repeat extra domain A of fibronectin; or 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, or induced target genes including e.g. Beta-Defensin, cell surface proteins; or human adjuvant proteins including trif, flt-3 ligand, Gp96 or fibronectin, etc., or any species homolog of any of the above human adjuvant proteins. Furthermore HGMB1 may be used as adjuvant protein.

Mammalian, in particular human adjuvant proteins may furthermore comprise cytokines which induce or enhance an innate immune response, including IL-1 alpha, IL1 beta, IL-2, IL-6, IL-7, IL-8, IL-9, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, IL-21, IL-23, TNFalpha, IFNalpha, IFNbeta, IFNgamma, GM-CSF, G-CSF, M-CSF; chemokines including IL-8, IP-10, MCP-1, MIP-1alpha, RANTES, Eotaxin, CCL21;

cytokines which are released from macrophages, including IL-1, IL-6, IL-8, IL-12 and TNF-alpha; as well as IL-1R1 and IL-1 alpha.

Therefore in this context it particularly preferred that the at least one RNA encodes at least one innate immune activator, preferably an adjuvant protein, more preferably a human adjuvant protein, or a fragment or variant thereof.

In this context it is particularly preferred that I constitutive active variant of an adjuvant protein is encoded by the at least one RNA, preferably a constitutive active variant of RIG-1 (ΔRIGI).

In another preferred embodiment the at least one RNA encodes HGMB1 as an innate immune activator, or a fragment or variant thereof.

According to preferred embodiments in the context of the present invention innate immune activators may be selected from any innate immune activator selected from the group consisting of CD55; Akt; ATF2; C1QBP; C1QC; Cardif; CCL11; CCL2; CCL21; CCL3; CCL5; CD59,Beta-Defensin; Cdc42; CFAD; CFAH; CFAI; CH60; CIITA; c-Jun; c-myc; CO8A; CO8B; CO8G; complement_system_component_C1INH; complement_system_component_C1qR; complement_system_component_C1s; complement_system_component_C4bp; complement_system_component_C6; complement_system_component_C7; complement_system_component_C8; complement_system_component_C9; complement_system_component_CR2; complement_system_component_CR3; complement_system_component_MASP-1; complement_system_component_MASP-2; complement_system_component_MBL; complement_system_component_MCP; CREB3; CREB3L1; CREB3L3; CREB3L4; CREB5; CRTC2; CXCL10; CXCL8; DJB11; DJB13; DJB14; DJC10; DJC12; DJC14; DJC15; DJC16; DJC17; DJC18; DJC22; DJC24; DJC25; DJC27; DJC28; DJC30; DNAJB12; DNAJC11; DNAJC21; DNJA1; DNJA2; DNJA3; DNJA4; DNJB1; DNJB2; DNJB3; DNJB4; DNJB5; DNJB6; DNJB7; DNJB8; DNJB9; DNJC1; DNJC2; DNJC3; DNJC4; DNJC5; DNJC7; DNJC8; DNJC9; Elk-1; ERK1; ERK2; Fibrinogen; fibronectin; FLT3_ligand; FOS; G-CSF; GM-CSF; GRP94_(gp96); GSK3A; GSK3B; HS71A; HS71B; HSC70; HSP10; HSP60; HSP70; HSP75; HSP90; HSP90B1; IFNalpha; IFNB; IFNG; IKK; IL-1; IL-1_alpha; IL-1_beta; IL-12; IL-13; IL-15; IL-16; IL-17A; IL-18; IL-1R1; IL-2; IL-21; IL-23; IL-6; IL-7; IL-9; IRAK1; IRAK2; IRAK4; IRF3; IRF-7; JNK; KPCB; KPCD; KPCD1; KPCD3; KPCE; KPCG; KPCI; KPCL; KPCT; KPCZ; I_IPAF; LGP2; M-CSF; MDA5; MK11; MK12; MK13; MK14; MKK1; MKK3; MKK4; MKK6; MKK7;

MSTP104; MyD88; NALP10; NALP11; NALP12; NALP13; NALP2; NALP3; NALP4; NALP5; NALP6; NALP7; NALP8; NALP9; NF-kappaB; NLRP14; NOD1; NOD2; NOD3; PI3K; PKD2; PKN1; PKN2; PKN3; PRKCA; PRKD2; Rab; Rac1; RASH; RASK; RASN; RhoA; RIG-I; Src-Kinases; Surfactant_protein_A; Surfactant_protein_D; TAK1; TBK1; TICAM1; TICAM2; TIRAP; TLR1; TLR10; TLR2; TLR3; TLR4; TLR5; TLR6; TLR7; TLR8; TLR9; TNF; TRAF6, preferably as disclosed in Table 11. Particularly preferred in this context are the RNA sequences encoding a innate immune activator according to Table 11.

Table 11: Innate immune activators (human adjuvant proteins)

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
CD55	UniProtKB: B1AP15	909	910	911, 912, 913, 914, 915
CD55	UniProtKB: D3DT85	916	917	918, 919, 920, 921, 922
CD55	UniProtKB: D3DT86	923	924	925, 926, 927, 928, 929
CD55	UniProtKB: P08174	930	931	932, 933, 934, 935, 936
fibronectin	UniProtKB: A0A024R5I6	1567	1568	1569, 1570, 1571, 1572, 1573
fibronectin	UniProtKB: A0A024RB01	1574	1575	1576, 1577, 1578, 1579, 1580
fibronectin	UniProtKB: A0A024RDT9	1581	1582	1583, 1584, 1585, 1586, 1587
fibronectin	UniProtKB: A0A024RDV5	1588	1589	1590, 1591, 1592, 1593, 1594
fibronectin	UniProtKB: A6NH44	1595	1596	1597, 1598, 1599, 1600, 1601
fibronectin	UniProtKB: A8K6A5	1602	1603	1604, 1605, 1606, 1607, 1608
fibronectin	UniProtKB: B2R627	1609	1610	1611, 1612, 1613, 1614, 1615
fibronectin	UniProtKB: B3KXM5	1616	1617	1618, 1619, 1620, 1621, 1622
fibronectin	UniProtKB: B4DIC5	1623	1624	1625, 1626, 1627, 1628, 1629
fibronectin	UniProtKB: B4DN21	1630	1631	1632, 1633, 1634, 1635, 1636
fibronectin	UniProtKB:	1637	1638	1639, 1640, 1641, 1642, 1643

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
	B4DS98			
fibronectin	UniProtKB: B4DTH2	1644	1645	1646, 1647, 1648, 1649, 1650
fibronectin	UniProtKB: B4DTK1	1651	1652	1653, 1654, 1655, 1656, 1657
fibronectin	UniProtKB: B4DU16	1658	1659	1660, 1661, 1662, 1663, 1664
fibronectin	UniProtKB: B7Z3W5	1665	1666	1667, 1668, 1669, 1670, 1671
fibronectin	UniProtKB: B7Z939	1672	1673	1674, 1675, 1676, 1677, 1678
fibronectin	UniProtKB: G5E9X3	1679	1680	1681, 1682, 1683, 1684, 1685
fibronectin	UniProtKB: Q9H382	1686	1687	1688, 1689, 1690, 1691, 1692
FOS	UniProtKB: P01100	1693	1694	1695, 1696, 1697, 1698, 1699
HS71A	UniProtKB: P0DMV8	1938	1939	1940, 1941, 1942, 1943, 1944
HS71B	UniProtKB: P0DMV9	1945	1946	1947, 1948, 1949, 1950, 1951
RASH	UniProtKB: P01112	3296	3297	3298, 3299, 3300, 3301, 3302
RASK	UniProtKB: P01116	3303	3304	3305, 3306, 3307, 3308, 3309
RASN	UniProtKB: P01111	3310	3311	3312, 3313, 3314, 3315, 3316
FLT3_ligand	Genbank: AAA90950.1	3913	3914	3915, 3916, 3917, 3918, 3919, 3920
FLT3_ligand	UniProtKB: P49771	3921	3922	3923, 3924, 3925, 3926, 3927, 3928
G-CSF	UniProtKB: P09919	3929	3930	3931, 3932, 3933, 3934, 3935, 3936
GM-CSF	UniProtKB: P04141	3945	3946	3947, 3948, 3949, 3950, 3951, 3952
IFNalpha	UniProtKB: G9JKF1	3953	3954	3955, 3956, 3957, 3958, 3959, 3960
IFNalpha	UniProtKB: P01562	3961	3962	3963, 3964, 3965, 3966, 3967, 3968
IFNalpha	UniProtKB: P01563	3969	3970	3971, 3972, 3973, 3974, 3975, 3976

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
IFNalpha	UniProtKB: P01566	3977	3978	3979, 3980, 3981, 3982, 3983, 3984
IFNalpha	UniProtKB: P01567	3985	3986	3987, 3988, 3989, 3990, 3991, 3992
IFNalpha	UniProtKB: P01568	3993	3994	3995, 3996, 3997, 3998, 3999, 4000
IFNalpha	UniProtKB: P01569	4001	4002	4003, 4004, 4005, 4006, 4007, 4008
IFNalpha	UniProtKB: P01570	4009	4010	4011, 4012, 4013, 4014, 4015, 4016
IFNalpha	UniProtKB: P01571	4017	4018	4019, 4020, 4021, 4022, 4023, 4024
IFNalpha	UniProtKB: P05013	4025	4026	4027, 4028, 4029, 4030, 4031, 4032
IFNalpha	UniProtKB: P05014	4033	4034	4035, 4036, 4037, 4038, 4039, 4040
IFNalpha	UniProtKB: P05015	4041	4042	4043, 4044, 4045, 4046, 4047, 4048
IFNalpha	UniProtKB: P32881	4049	4050	4051, 4052, 4053, 4054, 4055, 4056
IFNalpha	UniProtKB: Q14618	4057	4058	4059, 4060, 4061, 4062, 4063, 4064
IFNalpha	UniProtKB: Q86UP4	4065	4066	4067, 4068, 4069, 4070, 4071, 4072
IFNB	UniProtKB: P01574	4073	4074	4075, 4076, 4077, 4078, 4079, 4080
IFNB	UniProtKB: Q15943	4081	4082	4083, 4084, 4085, 4086, 4087, 4088
IFNG	UniProtKB: P01579	4089	4090	4091, 4092, 4093, 4094, 4095, 4096
IFNG	UniProtKB: Q14609	4097	4098	4099, 4100, 4101, 4102, 4103, 4104
IFNG	UniProtKB: Q14610	4105	4106	4107, 4108, 4109, 4110, 4111, 4112
IFNG	UniProtKB: Q14611	4113	4114	4115, 4116, 4117, 4118, 4119, 4120
IFNG	UniProtKB: Q14612	4121	4122	4123, 4124, 4125, 4126, 4127, 4128
IFNG	UniProtKB: Q14613	4129	4130	4131, 4132, 4133, 4134, 4135, 4136
IFNG	UniProtKB:	4137	4138	4139, 4140, 4141, 4142, 4143,

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
	Q14614			4144
IFNG	UniProtKB: Q14615	4145	4146	4147, 4148, 4149, 4150, 4151, 4152
IFNG	UniProtKB: Q8NHY9	4153	4154	4155, 4156, 4157, 4158, 4159, 4160
IL-12	UniProtKB: P29460	4193	4194	4195, 4196, 4197, 4198, 4199, 4200
IL-13	UniProtKB: P35225	4201	4202	4203, 4204, 4205, 4206, 4207, 4208
IL-15	UniProtKB: P40933	4217	4218	4219, 4220, 4221, 4222, 4223, 4224
IL-16	UniProtKB: Q14005	4225	4226	4227, 4228, 4229, 4230, 4231, 4232
IL-17A	UniProtKB: Q16552	4233	4234	4235, 4236, 4237, 4238, 4239, 4240
IL-18	UniProtKB: A0A024R3E0	4281	4282	4283, 4284, 4285, 4286, 4287, 4288
IL-18	UniProtKB: B0YJ28	4289	4290	4291, 4292, 4293, 4294, 4295, 4296
IL-18	UniProtKB: Q14116	4297	4298	4299, 4300, 4301, 4302, 4303, 4304
IL-1_alpha	UniProtKB: P01583	4313	4314	4315, 4316, 4317, 4318, 4319, 4320
IL-1_beta	UniProtKB: P01584	4321	4322	4323, 4324, 4325, 4326, 4327, 4328
IL-21	RefSeq: NP_001193935.1	4361	4362	4363, 4364, 4365, 4366, 4367, 4368
IL-21	RefSeq: NP_068575.1	4369	4370	4371, 4372, 4373, 4374, 4375, 4376
IL-23	UniProtKB: Q9NPF7	4385	4386	4387, 4388, 4389, 4390, 4391, 4392
IL-2	UniProtKB: P60568	4473	4474	4475, 4476, 4477, 4478, 4479, 4480
IL-2	UniProtKB: Q0GK43	4481	4482	4483, 4484, 4485, 4486, 4487, 4488
IL-2	UniProtKB: Q13169	4489	4490	4491, 4492, 4493, 4494, 4495, 4496
IL-2	UniProtKB: Q6NZ91	4497	4498	4499, 4500, 4501, 4502, 4503, 4504
IL-2	UniProtKB: Q6NZ93	4505	4506	4507, 4508, 4509, 4510, 4511, 4512

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
IL-6	UniProtKB: P05231	4593	4594	4595, 4596, 4597, 4598, 4599, 4600
IL-7	UniProtKB: A8K673	4601	4602	4603, 4604, 4605, 4606, 4607, 4608
IL-7	UniProtKB: P13232	4609	4610	4611, 4612, 4613, 4614, 4615, 4616
IL-9	UniProtKB: P15248	4617	4618	4619, 4620, 4621, 4622, 4623, 4624
M-CSF	UniProtKB: P09603	4705	4706	4707, 4708, 4709, 4710, 4711, 4712
CCL11	UniProtKB: P51671	4833	4834	4835, 4836, 4837, 4838, 4839, 4840
CCL11	UniProtKB: Q6I9T4	4841	4842	4843, 4844, 4845, 4846, 4847, 4848
CCL21	UniProtKB: O00585	4937	4938	4939, 4940, 4941, 4942, 4943, 4944
CCL2	UniProtKB: P13500	5001	5002	5003, 5004, 5005, 5006, 5007, 5008
CCL3	UniProtKB: A0N0R1	5009	5010	5011, 5012, 5013, 5014, 5015, 5016
CCL3	UniProtKB: P10147	5017	5018	5019, 5020, 5021, 5022, 5023, 5024
CCL5	UniProtKB: D0EI67	5041	5042	5043, 5044, 5045, 5046, 5047, 5048
CCL5	UniProtKB: P13501	5049	5050	5051, 5052, 5053, 5054, 5055, 5056
CXCL10	UniProtKB: A0A024RDA4	5129	5130	5131, 5132, 5133, 5134, 5135, 5136
CXCL10	UniProtKB: P02778	5137	5138	5139, 5140, 5141, 5142, 5143, 5144
CXCL8	UniProtKB: P10145	5265	5266	5267, 5268, 5269, 5270, 5271, 5272
TNF	UniProtKB: P01375	7369	7370	7371, 7372, 7373, 7374, 7375, 7376
TNF	UniProtKB: Q5STB3	7377	7378	7379, 7380, 7381, 7382, 7383, 7384
GRP94_(gp96)	UniProtKB: P14625	7617	7618	7619, 7620, 7621, 7622, 7623, 7624
HSC70	UniProtKB: P11142	7625	7626	7627, 7628, 7629, 7630, 7631, 7632
HSP60	UniProtKB:	7657	7658	7659, 7660, 7661, 7662, 7663,

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	A0A024R3X4			7664
HSP60	UniProtKB: B3GQS7	7665	7666	7667, 7668, 7669, 7670, 7671, 7672
HSP60	UniProtKB: P10809	7673	7674	7675, 7676, 7677, 7678, 7679, 7680
HSP60	UniProtKB: Q0VDF9	7681	7682	7683, 7684, 7685, 7686, 7687, 7688
HSP70	UniProtKB: P38646	7689	7690	7691, 7692, 7693, 7694, 7695, 7696
HSP90	UniProtKB: P07900	7697	7698	7699, 7700, 7701, 7702, 7703, 7704
HSP90	UniProtKB: P08238	7705	7706	7707, 7708, 7709, 7710, 7711, 7712
Akt	UniProtKB: B0LPE5	7737	7738	7739, 7740, 7741, 7742, 7743
Akt	UniProtKB: P31749	7744	7745	7746, 7747, 7748, 7749, 7750
Akt	UniProtKB: P31751	7751	7752	7753, 7754, 7755, 7756, 7757
Akt	UniProtKB: Q9Y243	7758	7759	7760, 7761, 7762, 7763, 7764
ATF2	UniProtKB: P15336	7765	7766	7767, 7768, 7769, 7770, 7771
C1QBP	UniProtKB: Q07021	7772	7773	7774, 7775, 7776, 7777, 7778
C1QC	UniProtKB: P02747	7779	7780	7781, 7782, 7783, 7784, 7785
Cardif	UniProtKB: Q7Z434	7786	7787	7788, 7789, 7790, 7791, 7792
CD59,Beta-Defensin	UniProtKB: P13987	7793	7794	7795, 7796, 7797, 7798, 7799
CD59,Beta-Defensin	UniProtKB: Q6FHM9	7800	7801	7802, 7803, 7804, 7805, 7806
Cdc42	UniProtKB: A0A024RAE4	7807	7808	7809, 7810, 7811, 7812, 7813
Cdc42	UniProtKB: A0A024RAE6	7814	7815	7816, 7817, 7818, 7819, 7820
Cdc42	UniProtKB: P60953	7821	7822	7823, 7824, 7825, 7826, 7827
CFAD	UniProtKB: P00746	7828	7829	7830, 7831, 7832, 7833, 7834

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CFAH	UniProtKB: P08603	7835	7836	7837, 7838, 7839, 7840, 7841
CFAI	UniProtKB: P05156	7842	7843	7844, 7845, 7846, 7847, 7848
CH60	RefSeq: NP_002147.2	7849	7850	7851, 7852, 7853, 7854, 7855
CIITA	UniProtKB: Q29704	7856	7857	7858, 7859, 7860, 7861, 7862
c-Jun	UniProtKB: B3KN68	7863	7864	7865, 7866, 7867, 7868, 7869
c-Jun	UniProtKB: B3KNW1	7870	7871	7872, 7873, 7874, 7875, 7876
c-Jun	UniProtKB: B3KXW5	7877	7878	7879, 7880, 7881, 7882, 7883
c-Jun	UniProtKB: B4DED9	7884	7885	7886, 7887, 7888, 7889, 7890
c-Jun	UniProtKB: B4DFU7	7891	7892	7893, 7894, 7895, 7896, 7897
c-Jun	UniProtKB: B4DGE1	7898	7899	7900, 7901, 7902, 7903, 7904
c-Jun	UniProtKB: B4DJ64	7905	7906	7907, 7908, 7909, 7910, 7911
c-Jun	UniProtKB: B4DS36	7912	7913	7914, 7915, 7916, 7917, 7918
c-Jun	UniProtKB: B7Z1L7	7919	7920	7921, 7922, 7923, 7924, 7925
c-Jun	UniProtKB: G1UI24	7926	7927	7928, 7929, 7930, 7931, 7932
c-Jun	UniProtKB: G5E966	7933	7934	7935, 7936, 7937, 7938, 7939
c-Jun	UniProtKB: O75843	7940	7941	7942, 7943, 7944, 7945, 7946
c-Jun	UniProtKB: P05412	7947	7948	7949, 7950, 7951, 7952, 7953
c-Jun	UniProtKB: P53677	7954	7955	7956, 7957, 7958, 7959, 7960
c-Jun	UniProtKB: P61966	7961	7962	7963, 7964, 7965, 7966, 7967
c-Jun	UniProtKB: Q63HQ0	7968	7969	7970, 7971, 7972, 7973, 7974
c-Jun	UniProtKB:	7975	7976	7977, 7978, 7979, 7980, 7981

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
	Q7Z5Q8			
c-Jun	UniProtKB: Q96PC3	7982	7983	7984, 7985, 7986, 7987, 7988
c-Jun	UniProtKB: Q9BXS5	7989	7990	7991, 7992, 7993, 7994, 7995
c-Jun	UniProtKB: Q9Y6Q5	7996	7997	7998, 7999, 8000, 8001, 8002
CO8A	UniProtKB: P07357	8003	8004	8005, 8006, 8007, 8008, 8009
CO8B	UniProtKB: P07358	8010	8011	8012, 8013, 8014, 8015, 8016
CO8G	UniProtKB: P07360	8017	8018	8019, 8020, 8021, 8022, 8023
complement_system_component_C1INH	UniProtKB: P05155	8024	8025	8026, 8027, 8028, 8029, 8030
complement_system_component_C1qR	UniProtKB: Q8IXK1	8031	8032	8033, 8034, 8035, 8036, 8037
complement_system_component_C1s	UniProtKB: P09871	8038	8039	8040, 8041, 8042, 8043, 8044
complement_system_component_C4bp	UniProtKB: P04003	8045	8046	8047, 8048, 8049, 8050, 8051
complement_system_component_C6	UniProtKB: P13671	8052	8053	8054, 8055, 8056, 8057, 8058
complement_system_component_C7	UniProtKB: P10643	8059	8060	8061, 8062, 8063, 8064, 8065
complement_system_component_C8	UniProtKB: Q99618	8066	8067	8068, 8069, 8070, 8071, 8072
complement_system_component_C9	UniProtKB: A0A024R035	8073	8074	8075, 8076, 8077, 8078, 8079
complement_system_component_C9	UniProtKB: P02748	8080	8081	8082, 8083, 8084, 8085, 8086
complement_system_component_CR2	UniProtKB: P20023	8087	8088	8089, 8090, 8091, 8092, 8093
complement_system_component_CR3	UniProtKB: D3DSM0	8094	8095	8096, 8097, 8098, 8099, 8100
complement_system_component_CR3	UniProtKB: P05107	8101	8102	8103, 8104, 8105, 8106, 8107
complement_system_component_MASP-1	UniProtKB: P48740	8108	8109	8110, 8111, 8112, 8113, 8114
complement_system_component_MASP-2	UniProtKB: O00187	8115	8116	8117, 8118, 8119, 8120, 8121

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
complement_system_component_MBL	UniProtKB: P11226	8122	8123	8124, 8125, 8126, 8127, 8128
complement_system_component_MCP	UniProtKB: P15529	8129	8130	8131, 8132, 8133, 8134, 8135
complement_system_component_MCP	UniProtKB: P40121	8136	8137	8138, 8139, 8140, 8141, 8142
CREB3	CCDS: CCDS6588.1	8143	8144	8145, 8146, 8147, 8148, 8149
CREB3L1	UniProtKB: Q96BA8	8150	8151	8152, 8153, 8154, 8155, 8156
CREB3L3	UniProtKB: Q68CJ9	8157	8158	8159, 8160, 8161, 8162, 8163
CREB3L4	UniProtKB: Q8TEY5	8164	8165	8166, 8167, 8168, 8169, 8170
CREB5	UniProtKB: Q02930	8171	8172	8173, 8174, 8175, 8176, 8177
CRTC2	UniProtKB: Q53ET0	8178	8179	8180, 8181, 8182, 8183, 8184
DJB11	UniProtKB: Q9UBS4	8185	8186	8187, 8188, 8189, 8190, 8191
DJB13	UniProtKB: P59910	8192	8193	8194, 8195, 8196, 8197, 8198
DJB14	UniProtKB: Q8TBM8	8199	8200	8201, 8202, 8203, 8204, 8205
DJC10	UniProtKB: Q8IXB1	8206	8207	8208, 8209, 8210, 8211, 8212
DJC12	UniProtKB: Q9UKB3	8213	8214	8215, 8216, 8217, 8218, 8219
DJC14	UniProtKB: Q6Y2X3	8220	8221	8222, 8223, 8224, 8225, 8226
DJC15	UniProtKB: Q9Y5T4	8227	8228	8229, 8230, 8231, 8232, 8233
DJC16	UniProtKB: Q9Y2G8	8234	8235	8236, 8237, 8238, 8239, 8240
DJC17	UniProtKB: Q9NVM6	8241	8242	8243, 8244, 8245, 8246, 8247
DJC18	UniProtKB: Q9H819	8248	8249	8250, 8251, 8252, 8253, 8254
DJC22	UniProtKB: Q8N4W6	8255	8256	8257, 8258, 8259, 8260, 8261
DJC24	UniProtKB:	8262	8263	8264, 8265, 8266, 8267, 8268

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
	Q6P3W2			
DJC25	UniProtKB: Q9H1X3	8269	8270	8271, 8272, 8273, 8274, 8275
DJC27	UniProtKB: Q9NZQ0	8276	8277	8278, 8279, 8280, 8281, 8282
DJC28	UniProtKB: Q9NX36	8283	8284	8285, 8286, 8287, 8288, 8289
DJC30	UniProtKB: Q96LL9	8290	8291	8292, 8293, 8294, 8295, 8296
DNAJB12	RefSeq: NP_001002762.2	8297	8298	8299, 8300, 8301, 8302, 8303
DNAJC11	UniProtKB: Q9NVH1	8304	8305	8306, 8307, 8308, 8309, 8310
DNAJC21	UniProtKB: Q5F1R6	8311	8312	8313, 8314, 8315, 8316, 8317
DNJA1	UniProtKB: P31689	8318	8319	8320, 8321, 8322, 8323, 8324
DNJA2	UniProtKB: O60884	8325	8326	8327, 8328, 8329, 8330, 8331
DNJA3	UniProtKB: Q96EY1	8332	8333	8334, 8335, 8336, 8337, 8338
DNJA4	UniProtKB: Q8WW22	8339	8340	8341, 8342, 8343, 8344, 8345
DNJB1	UniProtKB: P25685	8346	8347	8348, 8349, 8350, 8351, 8352
DNJB2	UniProtKB: P25686	8353	8354	8355, 8356, 8357, 8358, 8359
DNJB3	UniProtKB: Q8WWF6	8360	8361	8362, 8363, 8364, 8365, 8366
DNJB4	UniProtKB: Q9UDY4	8367	8368	8369, 8370, 8371, 8372, 8373
DNJB5	UniProtKB: O75953	8374	8375	8376, 8377, 8378, 8379, 8380
DNJB6	UniProtKB: O75190	8381	8382	8383, 8384, 8385, 8386, 8387
DNJB7	UniProtKB: Q7Z6W7	8388	8389	8390, 8391, 8392, 8393, 8394
DNJB8	UniProtKB: Q8NHS0	8395	8396	8397, 8398, 8399, 8400, 8401
DNJB9	UniProtKB: Q9UBS3	8402	8403	8404, 8405, 8406, 8407, 8408

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DNJC1	UniProtKB: Q96KC8	8409	8410	8411, 8412, 8413, 8414, 8415
DNJC2	UniProtKB: Q99543	8416	8417	8418, 8419, 8420, 8421, 8422
DNJC3	UniProtKB: Q13217	8423	8424	8425, 8426, 8427, 8428, 8429
DNJC4	UniProtKB: Q9NNZ3	8430	8431	8432, 8433, 8434, 8435, 8436
DNJC5	UniProtKB: Q9H3Z4	8437	8438	8439, 8440, 8441, 8442, 8443
DNJC7	UniProtKB: Q99615	8444	8445	8446, 8447, 8448, 8449, 8450
DNJC8	UniProtKB: O75937	8451	8452	8453, 8454, 8455, 8456, 8457
DNJC9	UniProtKB: Q8WXX5	8458	8459	8460, 8461, 8462, 8463, 8464
Elk-1	UniProtKB: P19419	8465	8466	8467, 8468, 8469, 8470, 8471
Elk-1	UniProtKB: Q8N9S0	8472	8473	8474, 8475, 8476, 8477, 8478
ERK1	UniProtKB: P27361	8479	8480	8481, 8482, 8483, 8484, 8485
ERK2	UniProtKB: P28482	8486	8487	8488, 8489, 8490, 8491, 8492
Fibrinogen	UniProtKB: A0A024R8B4	8493	8494	8495, 8496, 8497, 8498, 8499
Fibrinogen	UniProtKB: A4D1B8	8500	8501	8502, 8503, 8504, 8505, 8506
Fibrinogen	UniProtKB: A8K8X4	8507	8508	8509, 8510, 8511, 8512, 8513
Fibrinogen	UniProtKB: B4DTN2	8514	8515	8516, 8517, 8518, 8519, 8520
Fibrinogen	UniProtKB: B4E1D3	8521	8522	8523, 8524, 8525, 8526, 8527
Fibrinogen	UniProtKB: D3DP13	8528	8529	8530, 8531, 8532, 8533, 8534
Fibrinogen	UniProtKB: D3DP16	8535	8536	8537, 8538, 8539, 8540, 8541
Fibrinogen	UniProtKB: D3DSP9	8542	8543	8544, 8545, 8546, 8547, 8548
Fibrinogen	UniProtKB:	8549	8550	8551, 8552, 8553, 8554, 8555

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	P02671			
Fibrinogen	UniProtKB: P02675	8556	8557	8558, 8559, 8560, 8561, 8562
Fibrinogen	UniProtKB: P02679	8563	8564	8565, 8566, 8567, 8568, 8569
Fibrinogen	UniProtKB: Q08830	8570	8571	8572, 8573, 8574, 8575, 8576
Fibrinogen	UniProtKB: Q14314	8577	8578	8579, 8580, 8581, 8582, 8583
Fibrinogen	UniProtKB: Q6UXM4	8584	8585	8586, 8587, 8588, 8589, 8590
Fibrinogen	UniProtKB: Q9UE34	8591	8592	8593, 8594, 8595, 8596, 8597
FOS	UniProtKB: A0A024RD16	8598	8599	8600, 8601, 8602, 8603, 8604
GSK3A	UniProtKB: P49840	8605	8606	8607, 8608, 8609, 8610, 8611
GSK3B	UniProtKB: P49841	8612	8613	8614, 8615, 8616, 8617, 8618
HSP10	UniProtKB: P61604	8619	8620	8621, 8622, 8623, 8624, 8625
HSP75	UniProtKB: Q12931	8626	8627	8628, 8629, 8630, 8631, 8632
HSP90B1	UniProtKB: Q5CAQ5	8633	8634	8635, 8636, 8637, 8638, 8639
IKK	UniProtKB: O14920	8640	8641	8642, 8643, 8644, 8645, 8646
IKK	UniProtKB: Q14164	8647	8648	8649, 8650, 8651, 8652, 8653
IKK	UniProtKB: Q9Y6K9	8654	8655	8656, 8657, 8658, 8659, 8660
IL-1	UniProtKB: O43353	8661	8662	8663, 8664, 8665, 8666, 8667
IL-1	UniProtKB: Q8N9C1	8668	8669	8670, 8671, 8672, 8673, 8674
IL-1	UniProtKB: Q8WWZ1	8675	8676	8677, 8678, 8679, 8680, 8681
IL-1	UniProtKB: Q9NZH7	8682	8683	8684, 8685, 8686, 8687, 8688
IL-1	UniProtKB: Q9UBH0	8689	8690	8691, 8692, 8693, 8694, 8695

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IL-1	UniProtKB: Q9UHA7	8696	8697	8698, 8699, 8700, 8701, 8702
IL-1R1	UniProtKB: P14778	8703	8704	8705, 8706, 8707, 8708, 8709
IL-1R1	UniProtKB: Q6NWP5	8710	8711	8712, 8713, 8714, 8715, 8716
IL-1R1	UniProtKB: Q6NWP6	8717	8718	8719, 8720, 8721, 8722, 8723
IRAK1	UniProtKB: L8E7M9	8724	8725	8726, 8727, 8728, 8729, 8730
IRAK1	UniProtKB: P51617	8731	8732	8733, 8734, 8735, 8736, 8737
IRAK2	UniProtKB: O43187	8738	8739	8740, 8741, 8742, 8743, 8744
IRAK4	UniProtKB: Q69FE3	8745	8746	8747, 8748, 8749, 8750, 8751
IRAK4	UniProtKB: Q7Z6A7	8752	8753	8754, 8755, 8756, 8757, 8758
IRAK4	UniProtKB: Q7Z6A8	8759	8760	8761, 8762, 8763, 8764, 8765
IRAK4	UniProtKB: Q9NWZ3	8766	8767	8768, 8769, 8770, 8771, 8772
IRF3	UniProtKB: A0A024QZE1	8773	8774	8775, 8776, 8777, 8778, 8779
IRF3	UniProtKB: E2GIM5	8780	8781	8782, 8783, 8784, 8785, 8786
IRF3	UniProtKB: E2GIM6	8787	8788	8789, 8790, 8791, 8792, 8793
IRF3	UniProtKB: E2GIM7	8794	8795	8796, 8797, 8798, 8799, 8800
IRF3	UniProtKB: E2GIM8	8801	8802	8803, 8804, 8805, 8806, 8807
IRF3	UniProtKB: E2GIM9	8808	8809	8810, 8811, 8812, 8813, 8814
IRF3	UniProtKB: Q14653	8815	8816	8817, 8818, 8819, 8820, 8821
IRF3	UniProtKB: Q96GL3	8822	8823	8824, 8825, 8826, 8827, 8828
IRF-7	UniProtKB: Q92985	8829	8830	8831, 8832, 8833, 8834, 8835
JNK	UniProtKB:	8836	8837	8838, 8839, 8840, 8841, 8842

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	B4DU99			
KPCB	UniProtKB: P05771-1	8843	8844	8845, 8846, 8847, 8848, 8849
KPCB	UniProtKB: P05771-2	8850	8851	8852, 8853, 8854, 8855, 8856
KPCD1	UniProtKB: Q15139	8857	8858	8859, 8860, 8861, 8862, 8863
KPCD3	UniProtKB: O94806	8864	8865	8866, 8867, 8868, 8869, 8870
KPCD	UniProtKB: Q05655	8871	8872	8873, 8874, 8875, 8876, 8877
KPCE	UniProtKB: Q02156	8878	8879	8880, 8881, 8882, 8883, 8884
KPCG	UniProtKB: P05129	8885	8886	8887, 8888, 8889, 8890, 8891
KPCI	UniProtKB: P41743	8892	8893	8894, 8895, 8896, 8897, 8898
KPCL	UniProtKB: P24723	8899	8900	8901, 8902, 8903, 8904, 8905
KPCT	UniProtKB: Q04759	8906	8907	8908, 8909, 8910, 8911, 8912
KPCZ	UniProtKB: Q05513	8913	8914	8915, 8916, 8917, 8918, 8919
LGP2	UniProtKB: A0A024R1Y5	8920	8921	8922, 8923, 8924, 8925, 8926
LGP2	UniProtKB: Q96C10	8927	8928	8929, 8930, 8931, 8932, 8933
l_IPAF	UniProtKB: Q9NPP4	8934	8935	8936, 8937, 8938, 8939, 8940
MDA5	UniProtKB: Q9BYX4	8941	8942	8943, 8944, 8945, 8946, 8947
MK11	UniProtKB: Q15759	8948	8949	8950, 8951, 8952, 8953, 8954
MK12	UniProtKB: P53778	8955	8956	8957, 8958, 8959, 8960, 8961
MK13	UniProtKB: O15264	8962	8963	8964, 8965, 8966, 8967, 8968
MK14	UniProtKB: Q16539	8969	8970	8971, 8972, 8973, 8974, 8975
MKK1	UniProtKB: Q02750	8976	8977	8978, 8979, 8980, 8981, 8982

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
MKK3	UniProtKB: P46734	8983	8984	8985, 8986, 8987, 8988, 8989
MKK4	UniProtKB: P45985	8990	8991	8992, 8993, 8994, 8995, 8996
MKK6	UniProtKB: P52564	8997	8998	8999, 9000, 9001, 9002, 9003
MKK7	UniProtKB: O14733	9004	9005	9006, 9007, 9008, 9009, 9010
MSTP104	UniProtKB: Q7Z4D5	9011	9012	9013, 9014, 9015, 9016, 9017
MyD88	UniProtKB: Q99836	9018	9019	9020, 9021, 9022, 9023, 9024
NALP10	UniProtKB: Q86W26	9025	9026	9027, 9028, 9029, 9030, 9031
NALP11	UniProtKB: P59045	9032	9033	9034, 9035, 9036, 9037, 9038
NALP12	UniProtKB: P59046	9039	9040	9041, 9042, 9043, 9044, 9045
NALP13	UniProtKB: Q86W25	9046	9047	9048, 9049, 9050, 9051, 9052
NALP2	UniProtKB: Q8WY49	9053	9054	9055, 9056, 9057, 9058, 9059
NALP2	UniProtKB: Q9NX02	9060	9061	9062, 9063, 9064, 9065, 9066
NALP3	UniProtKB: Q96P20	9067	9068	9069, 9070, 9071, 9072, 9073
NALP4	UniProtKB: Q96MN2	9074	9075	9076, 9077, 9078, 9079, 9080
NALP5	UniProtKB: P59047	9081	9082	9083, 9084, 9085, 9086, 9087
NALP6	UniProtKB: P59044	9088	9089	9090, 9091, 9092, 9093, 9094
NALP7	UniProtKB: Q8WX94	9095	9096	9097, 9098, 9099, 9100, 9101
NALP8	UniProtKB: Q86W28	9102	9103	9104, 9105, 9106, 9107, 9108
NALP9	UniProtKB: Q7RTR0	9109	9110	9111, 9112, 9113, 9114, 9115
NF-kappaB	UniProtKB: A3F768	9116	9117	9118, 9119, 9120, 9121, 9122
NF-kappaB	UniProtKB:	9123	9124	9125, 9126, 9127, 9128, 9129

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
	A3F769			
NLRP14	UniProtKB: Q86UT6	9130	9131	9132, 9133, 9134, 9135, 9136
NLRP14	UniProtKB: Q86W24	9137	9138	9139, 9140, 9141, 9142, 9143
NOD1	UniProtKB: G3XAL1	9144	9145	9146, 9147, 9148, 9149, 9150
NOD1	UniProtKB: Q9Y239	9151	9152	9153, 9154, 9155, 9156, 9157
NOD2	UniProtKB: Q9HC29	9158	9159	9160, 9161, 9162, 9163, 9164
NOD3	UniProtKB: C3VPR7	9165	9166	9167, 9168, 9169, 9170, 9171
NOD3	UniProtKB: H3BLT9	9172	9173	9174, 9175, 9176, 9177, 9178
NOD3	UniProtKB: Q7RTR2	9179	9180	9181, 9182, 9183, 9184, 9185
PI3K	UniProtKB: O00329	9186	9187	9188, 9189, 9190, 9191, 9192
PI3K	UniProtKB: O00459	9193	9194	9195, 9196, 9197, 9198, 9199
PI3K	UniProtKB: P27986	9200	9201	9202, 9203, 9204, 9205, 9206
PI3K	UniProtKB: P42336	9207	9208	9209, 9210, 9211, 9212, 9213
PI3K	UniProtKB: P42338	9214	9215	9216, 9217, 9218, 9219, 9220
PI3K	UniProtKB: P48736	9221	9222	9223, 9224, 9225, 9226, 9227
PI3K	UniProtKB: Q5UE93	9228	9229	9230, 9231, 9232, 9233, 9234
PI3K	UniProtKB: Q8NEB9	9235	9236	9237, 9238, 9239, 9240, 9241
PI3K	UniProtKB: Q8WYR1	9242	9243	9244, 9245, 9246, 9247, 9248
PKD2	UniProtKB: Q13563	9249	9250	9251, 9252, 9253, 9254, 9255
PKN1	UniProtKB: Q16512	9256	9257	9258, 9259, 9260, 9261, 9262
PKN2	UniProtKB: Q16513	9263	9264	9265, 9266, 9267, 9268, 9269

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
PKN3	UniProtKB: Q6P5Z2	9270	9271	9272, 9273, 9274, 9275, 9276
PRKCA	UniProtKB: P17252	9277	9278	9279, 9280, 9281, 9282, 9283
PRKD2	RefSeq: NP_001073349.1	9284	9285	9286, 9287, 9288, 9289, 9290
Rab	UniProtKB: P52594	9291	9292	9293, 9294, 9295, 9296, 9297
Rac1	UniProtKB: A4D2P0	9298	9299	9300, 9301, 9302, 9303, 9304
Rac1	UniProtKB: A4D2P1	9305	9306	9307, 9308, 9309, 9310, 9311
Rac1	UniProtKB: A4D2P2	9312	9313	9314, 9315, 9316, 9317, 9318
Rac1	UniProtKB: P63000	9319	9320	9321, 9322, 9323, 9324, 9325
Rac1	UniProtKB: W0UV93	9326	9327	9328, 9329, 9330, 9331, 9332
RhoA	UniProtKB: A0A024R324	9333	9334	9335, 9336, 9337, 9338, 9339
RhoA	UniProtKB: P61586	9340	9341	9342, 9343, 9344, 9345, 9346
RIG-I	UniProtKB: O95786	9347	9348	9349, 9350, 9351, 9352, 9353
RIG-I	UniProtKB: Q8IUD6	9354	9355	9356, 9357, 9358, 9359, 9360
Src-Kinases	UniProtKB: Q9H5V8	9361	9362	9363, 9364, 9365, 9366, 9367
Surfactant_protein_A	UniProtKB: Q8IWL1	9368	9369	9370, 9371, 9372, 9373, 9374
Surfactant_protein_A	UniProtKB: Q8IWL2	9375	9376	9377, 9378, 9379, 9380, 9381
Surfactant_protein_D	UniProtKB: P35247	9382	9383	9384, 9385, 9386, 9387, 9388
TAK1	UniProtKB: O43318	9389	9390	9391, 9392, 9393, 9394, 9395
TAK1	UniProtKB: P49116	9396	9397	9398, 9399, 9400, 9401, 9402
TBK1	UniProtKB: Q9UHD2	9403	9404	9405, 9406, 9407, 9408, 9409
TICAM1	UniProtKB:	9410	9411	9412, 9413, 9414, 9415, 9416

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
	Q8IUC6			
TICAM2	UniProtKB: Q86XR7	9417	9418	9419, 9420, 9421, 9422, 9423
TIRAP	UniProtKB: A0A024R3M4	9424	9425	9426, 9427, 9428, 9429, 9430
TIRAP	UniProtKB: P58753	9431	9432	9433, 9434, 9435, 9436, 9437
TLR10	UniProtKB: A0A024R9W4	9438	9439	9440, 9441, 9442, 9443, 9444
TLR10	UniProtKB: D1CS19	9445	9446	9447, 9448, 9449, 9450, 9451
TLR10	UniProtKB: D1CS20	9452	9453	9454, 9455, 9456, 9457, 9458
TLR10	UniProtKB: D1CS24	9459	9460	9461, 9462, 9463, 9464, 9465
TLR10	UniProtKB: D1CS26	9466	9467	9468, 9469, 9470, 9471, 9472
TLR10	UniProtKB: D1CS27	9473	9474	9475, 9476, 9477, 9478, 9479
TLR10	UniProtKB: D1CS28	9480	9481	9482, 9483, 9484, 9485, 9486
TLR10	UniProtKB: D1CS29	9487	9488	9489, 9490, 9491, 9492, 9493
TLR10	UniProtKB: D1CS30	9494	9495	9496, 9497, 9498, 9499, 9500
TLR10	UniProtKB: Q9BXR5	9501	9502	9503, 9504, 9505, 9506, 9507
TLR1	UniProtKB: D1CS34	9508	9509	9510, 9511, 9512, 9513, 9514
TLR1	UniProtKB: D1CS35	9515	9516	9517, 9518, 9519, 9520, 9521
TLR1	UniProtKB: D1CS36	9522	9523	9524, 9525, 9526, 9527, 9528
TLR1	UniProtKB: D1CS38	9529	9530	9531, 9532, 9533, 9534, 9535
TLR1	UniProtKB: D1CS42	9536	9537	9538, 9539, 9540, 9541, 9542
TLR1	UniProtKB: D1CS43	9543	9544	9545, 9546, 9547, 9548, 9549
TLR1	UniProtKB: D1CS44	9550	9551	9552, 9553, 9554, 9555, 9556

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
TLR1	UniProtKB: Q15399	9557	9558	9559, 9560, 9561, 9562, 9563
TLR1	UniProtKB: Q5FWG5	9564	9565	9566, 9567, 9568, 9569, 9570
TLR1	UniProtKB: Q6FI64	9571	9572	9573, 9574, 9575, 9576, 9577
TLR2	UniProtKB: O60603	9578	9579	9580, 9581, 9582, 9583, 9584
TLR3	UniProtKB: O15455	9585	9586	9587, 9588, 9589, 9590, 9591
TLR4	UniProtKB: D1CS55	9592	9593	9594, 9595, 9596, 9597, 9598
TLR4	UniProtKB: O00206	9599	9600	9601, 9602, 9603, 9604, 9605
TLR5	UniProtKB: D1CS79	9606	9607	9608, 9609, 9610, 9611, 9612
TLR5	UniProtKB: D1CS82	9613	9614	9615, 9616, 9617, 9618, 9619
TLR5	UniProtKB: D1CS83	9620	9621	9622, 9623, 9624, 9625, 9626
TLR5	UniProtKB: D1CS84	9627	9628	9629, 9630, 9631, 9632, 9633
TLR5	UniProtKB: D1CS85	9634	9635	9636, 9637, 9638, 9639, 9640
TLR5	UniProtKB: D1CS87	9641	9642	9643, 9644, 9645, 9646, 9647
TLR5	UniProtKB: D1CS88	9648	9649	9650, 9651, 9652, 9653, 9654
TLR5	UniProtKB: D1CS89	9655	9656	9657, 9658, 9659, 9660, 9661
TLR5	UniProtKB: D1CS90	9662	9663	9664, 9665, 9666, 9667, 9668
TLR6	UniProtKB: B6CH37	9669	9670	9671, 9672, 9673, 9674, 9675
TLR6	UniProtKB: B6CH42	9676	9677	9678, 9679, 9680, 9681, 9682
TLR6	UniProtKB: B6CH44	9683	9684	9685, 9686, 9687, 9688, 9689
TLR6	UniProtKB: B6CH45	9690	9691	9692, 9693, 9694, 9695, 9696
TLR6	UniProtKB:	9697	9698	9699, 9700, 9701, 9702, 9703

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
	B6RFS7			
TLR6	UniProtKB: D1CS91	9704	9705	9706, 9707, 9708, 9709, 9710
TLR6	UniProtKB: D1CS92	9711	9712	9713, 9714, 9715, 9716, 9717
TLR6	UniProtKB: D1CS93	9718	9719	9720, 9721, 9722, 9723, 9724
TLR6	UniProtKB: D1CS96	9725	9726	9727, 9728, 9729, 9730, 9731
TLR6	UniProtKB: D1CS97	9732	9733	9734, 9735, 9736, 9737, 9738
TLR6	UniProtKB: D1CS98	9739	9740	9741, 9742, 9743, 9744, 9745
TLR6	UniProtKB: D1CS99	9746	9747	9748, 9749, 9750, 9751, 9752
TLR6	UniProtKB: D1CSA0	9753	9754	9755, 9756, 9757, 9758, 9759
TLR7	UniProtKB: B2R9N9	9760	9761	9762, 9763, 9764, 9765, 9766
TLR7	UniProtKB: D1CS68	9767	9768	9769, 9770, 9771, 9772, 9773
TLR7	UniProtKB: Q9NYK1	9774	9775	9776, 9777, 9778, 9779, 9780
TLR8	UniProtKB: Q495P6	9781	9782	9783, 9784, 9785, 9786, 9787
TLR8	UniProtKB: Q495P7	9788	9789	9790, 9791, 9792, 9793, 9794
TLR8	UniProtKB: Q9NR97	9795	9796	9797, 9798, 9799, 9800, 9801
TLR9	UniProtKB: B6CH46	9802	9803	9804, 9805, 9806, 9807, 9808
TLR9	UniProtKB: D1CS61	9809	9810	9811, 9812, 9813, 9814, 9815
TLR9	UniProtKB: D1CS62	9816	9817	9818, 9819, 9820, 9821, 9822
TLR9	UniProtKB: L0R5D6	9823	9824	9825, 9826, 9827, 9828, 9829
TLR9	UniProtKB: L8E8B9	9830	9831	9832, 9833, 9834, 9835, 9836
TLR9	UniProtKB: Q9NR96	9837	9838	9839, 9840, 9841, 9842, 9843

Gene Name	Protein Accession No.	Protein Sequence SEQ ID NO:	RNA Sequence wild type SEQ ID NO:	Optimized RNA Sequence SEQ ID NO:
TRAF6	UniProtKB: Q9Y4K3	9844	9845	9846, 9847, 9848, 9849, 9850
c-myc	UniProtKB: A0A0B4J1R1	9851	9852	9853, 9854, 9855, 9856, 9857, 9858
c-myc	UniProtKB: P01106	9859	9860	9861, 9862, 9863, 9864, 9865, 9866
c-myc	UniProtKB: Q14901	9867	9868	9869, 9870, 9871, 9872, 9873, 9874
c-myc	UniProtKB: Q16591	9875	9876	9877, 9878, 9879, 9880, 9881, 9882

According to the present invention, in a more preferred embodiment, the inventive composition comprises at least one RNA, preferably an mRNA comprising at least one coding region encoding at least one innate immune activator or a fragment or variant thereof, wherein the at least one coding region comprises an RNA sequence being identical or at least 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the RNA sequences according to the SEQ ID Nos as disclosed in Table 11.

13. Antibodies, decoy receptors and dominant negative receptors:

According to a preferred embodiment the at least one RNA of the inventive RNA containing composition encodes at least one antibody and/or at least one dominant negative receptor and/or at least one decoy receptor or a fragment or variant thereof, modulating (e.g. inhibiting) the functionality of a protein or signaling pathway which is associated with tumor or cancer development. It is particularly preferred that the RNA containing composition comprises at least one RNA encoding the heavy chain of a particular antibody or fragments or variants thereof and at least one further RNA encoding the light chain of the same particular antibody or fragments or variants thereof.

In this context particularly preferred are the antibodies according to Table 12.

Table 12: Antibodies directed against proteins accociated with tumor or cancer development

Name	Target
3F8	GD2
Abagovomab	CA-125 imitation
Abciximab	Platelet glycoprotein GPIIb/IIIa
Adecatumumab	EpCAM (CD326)
Afutuzumab	CD20
Alacizumab pegol	VEGFR2
Alemtuzumab	CD52
Altumomab pentetate	CEA
Amatuximab	mesothelin
Anatumomab mafenatox	5T4
Anetumab ravtansine	mesothelin
Apolizumab	HLA-DR beta
apomab	TRAIL-R2 (CD262)
Arcitumomab	CEA
Ascrinvacumab	ACVRL1
Bavituximab	phosphatidylserine
Bectumomab	CD22
Belimumab	BAFF
Besilesomab	CEA
Bevacizumab	VEGF-A
Bivatuzumab mertansine	CD44v6
Blinatumomab	CD19 x CD3
Brentuximab vedotin	CD30 (TNFRSF8)
Brontictuzumab	NOTCH1
canakinumab	IL-1β
Cantuzumab mertansine	CanAg
Cantuzumab ravtansine	MUC1 (CD227)
Capromab pendetide	PSMA

Name	Target
Carlumab	MCP-1
Catumaxomab	EpCAM x CD3
cBR-doxorubicin immunoconjugate	CD174 (Lewis Y)
Cetuximab	EGFR (HER1/ERBB1)
Citatuzumab bogatox	EpCAM
Cixutumumab	IGF-1R
Clivatuzumab tetraxetan	MUC1 (CD227)
Codrituzumab	glypican 3
Coltuximab ravtansine	CD19
Conatumumab	TRAIL-R2 (CD262)
Dacetuzumab	CD40
Dalotuzumab	IGF-1R
Dalotuzumab	insulin-like growth factor I receptor
Daratumumab	CD38 (cyclic ADP ribose hydrolase)
Demcizumab	DLL4
Denintuzumab mafodotin	CD19
Denosumab	RANKL
Depatuxizumab	EGFR (HER1/ERBB1)
Derlotuximab	histone complex
Detumomab	unknown (B-lymphoma cells)
Dinutuximab	B4GALNT1
Drozitumab	TRAIL-R2 (CD262)
Duligotumab	HER3 (ERBB3)
Duligotuzumab	EGFR (HER1/ERBB1)
Dusigitumab	ILGF2
Ecromeximab	GD3 ganglioside
Edrecolomab	EpCAM
Elgemtumab	ERBB3
Elotuzumab	SLAMF7 (CD319)

Name	Target
Elsilimomab	IL-6
Emactuzumab	CSF1R
Emibetuzumab	HGFR
Emibetuzumab	MET
Enavatuzumab	TNFRSF12A
Enfortumab vedotin	AGS-22M6
Enoticumab	DLL4
Ensituximab	MUC5AC
Epitumomab cituxetan	MUC1 (CD227)
Epratuzumab	CD22
Ertumaxomab	HER2 (ERBB2/neu) x CD3
Etaracizumab	integrin α5β3
Faralimomab	IFNA1
Farletuzumab	FOLR1 alpha
FBTA	CD20 x CD3
Ficlatuzumab	HGFR
Figitumumab	IGF-1R
Flanvotumab	TYRP1(glycoprotein 75)
Fresolimumab	TGF-β
Futuximab	EGFR (HER1/ERBB1)
Galiximab	CD80
Gantiumab	IGF-1R
Gemtuzumab ozogamicin	CD33
Girentuximab	Carbonic anhydrase 9 (CA9/CAIX)
Glembatumumab vedotin	GPNMB
glycooptimized trastuzumab-GEX	HER2 (ERBB2/neu)
Ibritumomab tiuxetan	CD20
Icrucumab	VEGFR-1
Igovomab	MUC16

Name	Target
IMAB362	Claudin-18 (CLDN18.2)
Imgatuzumab	EGFR (HER1/ERBB1)
Indatuximab ravtansine	SDC1
Indusatumab vedotin	GUCY2C
inebilizumab	CD19
Inotuzumab ozogamicin	CD22
Intetumumab	CD51
Iratumumab	CD30 (TNFRSF8)
Isatuximab	CD38
Labetuzumab	CEA
Lenzilumab	CSF2
Lexatumumab	TRAIL-R2 (CD262)
Lifastuzumab vedotin	NaPi2B
Lilotomab satetraxetan	CD37
Lintuzumab	CD33
Lorvotuzumab mertansine	CD56
Lucatumumab	CD40
Lumiliximab	CD23 (IgE receptor)
Lumretuzumab	ERBB3
Mapatumumab	TRAIL-R1 (CD261)
Margetuximab	HER2 (ERBB2/neu)
Matuzumab	EGFR (HER1/ERBB1)
Mepolizumab	IL-5
Milatuzumab	CD74
Minretumomab	TAG-72
Mirvetuximab soravtansine	FOLR1 alpha
Mitumomab	GD3 (ganglioside)
Mogamulizumab	CCR4
Moxetumomab pasudotox	CD22

Name	Target
Nacolomab tafenatox	C242 antigen
Naptumomab estafenatox	5T4
Narnatumab	RON
Necitumumab	EGFR (HER1/ERBB1)
Nesvacumab	ANGPT2 (angiopoietin 2)
Nimotuzumab	EGFR (HER1/ERBB1)
Nofetumomab merpentan	EpCAM
binutuzumab	CD20
Ocaratuzumab	CD20
Ofatumumab	CD20
Olaratumab	PDGFRα
Onartuzumab	MET
Ontuxizumab	CD248 (TEM1)
Oportuzumab monatox	EpCAM
Oregovomab	CA-125
Otlertuzumab	CD37
Panitumumab	EGFR (HER1/ERBB1)
Pankomab	MUC1 (tumor specific glycosylation)
Parsatuzumab	EGFL7
Pasotuxizumab	FOLH1
Patritumab	HER3 (ERBB3)
Pemtumomab	MUC1 (CD227)
Pertuzumab	HER2 (ERBB2/neu)
Pinatuzumab vedotin	CD22
Pintumomab	adenocarcinoma antigen
Polatuzumab vedotin	CD79B
Racotumomab	NGcGM3
Radretumab	EDB (fibronectin extra domain-B)
Ramucirumab	VEGFR2

Name	Target
Rilotumumab	HGFR
Rituximab	CD20
Robatumumab	IGF-1R
Sacituzumab govitecan	Trop-2 (tumor-associated calcium signal transducer 2/EGP-1)
Samalizumab	CD200 (OX-2 membrane glycoprotein)
Satumomab pendetide	TAG-72
Seribantumab	ERBB3
Seribantumab	HER3 (ERBB3)
SGN-CDA	CD19
SGN-CDA	CD33
Sibrotuzumab	FAP
Siltuximab	IL-6
Simtuzumab	LOXL2
Sofituzumab vedotin	CA 125
Solitomab	EpCAM
Sonepcizumab	S1P (sphingosine-1-phosphate)
Tacatuzumab tetraxetan	AFP (alpha-fetoprotein)
Taplitumomab paptox	CD19
Tarextumab	Notch receptor
Tenatumomab	TN-C (tenascin C)
Teprotumumab	CD221
Tetulomab	CD37
TGN	CD28
Tigatuzumab	TRAIL-R2 (CD262)
Lebrikizumab	IL-13
Tocilizumab	IL-6R
Tositumomab	CD20
Tovetumab	CD140a

Name	Target
Tovetumab	PDGFRα
Trastuzumab	HER2 (ERBB2/neu)
Trastuzumab emtansine	HER2 (ERBB2/neu)
TRBS	GD2
Tucotuzumab celmoleukin	EpCAM
ublituximab	CD20
Ublituximab	MS4A1
Ulocuplumab	CXCR4
Vandortuzumab vedotin	STEAP1
Vantictumab	FZD7
Vanucizumab	Ang-2 (angiopoietin 2) x VEGF-A
Veltuzumab	CD20
Vesencumab	NRP1
Volociximab	integrin α5β1
Votumumab	CTAA16.88
Zalutumumab	EGFR (HER1/ERBB1)
Zanolimumab	CD4
Zatuximab	HER1 (EGFR/ERBB1)

Preferably, the neutralizing antibody is chosen from the list of anti-IL-10 and anti-TGFbeta.

Furthermore, the at least one antibody may preferably chosen from anti-CD73 antibodies or fragments or variants thereof.

In a further particularly preferred embodiment the at least one antibody is chosen from an antibody directed against CCR5/CD195 or from an antibody directed against its ligand CCL5/RANTES.

In a particularly preferred embodiment the decoy receptor is a soluble CCR5 (chemokine receptor type 5, also known as CD195).

In a further particularly preferred embodiment the dominant negative receptor is dominant negative CCR5 (chemokine receptor type 5, also known as CD195).

Furthermore, the at least one antibody may preferably chosen from anti-CD73 antibodies or fragments or variants thereof.

14. Inhibitors of myeloid derived suppressor cells (MDSCs):

Myeloid Derived Suppressor Cells (MDSC) are a heterogeneous population of immature myeloid cells that are increased in cancer and related disorders. MDSC are induced by tumor secreted growth factors. MDSC play an important part in suppression of host immune responses through several mechanisms. In addition, MDSC may also contribute to angiogenesis and tumor invasion. Therefore, MDSC inhibition is a strategy for the treatment of cancer and related disorders.

In the context of the invention, MDSC inhibition can be achieved by direct deactivation of MDSCs (e.g., anti IL-17 antibodies), by blocking differentiation of MDSCs into mature cells (e.g., IL-12), by blocking the cell development of MDSCs or by depletion of MDSCs (e.g., cytotoxic agents).

Therefore it is particularly preferred to use anti IL-17 antibodies and IL-12 as inhibitors of MDSCs.

15. IDO pathway inhibitors

In a further preferred embodiment of the inventive RNA containing composition the RNA, preferably mRNA codes for at least one IDO pathway inhibitor. Preferably the RNA encoding the at least one IDO pathway inhibitor encodes an inhibitory protein or dominant negative mutant protein of the IDO pathway.

As reviewed in Prendergast et al. (Prendergast GC, Smith C, Thomas S, Mandik-Nayak L, Laury-Kleintop L, Metz R, Muller AJ. Indoleamine 2,3-dioxygenase pathways of pathogenic inflammation and immune escape in cancer. Cancer Immunol. Immunother. 2014 Jul;63(7):721-35) indoleamine-pyrrole 2,3-dioxygenase (IDO or INDO EC 1.13.11.52) is an enzyme that in humans is encoded by the IDO1 gene. This enzyme catalyzes the degradation of the essential amino acid L-tryptophan to N-formylkynurenine. IDO is the first and rate-limiting enzyme of tryptophan catabolism through kynurenine pathway, thus causing depletion of tryptophan which can cause halted growth of microbes as well as T cells. IDO is an immunomodulatory enzyme produced by some alternatively activated macrophages and other immunoregulatory cells (also used as an immune subversion strategy by many tumors). The clinical development of IDO inhibitors may produce a novel class of immunomodulators with broad application in the treatment of advanced human cancer.

16. Proteins or peptides that bind inhibitors of apoptosis

Apoptosis is a tightly regulated cellular process and faulty regulation of apoptosis is a hallmark of human cancers. Targeting key apoptosis regulators with the goal to restore apoptosis in tumor cells has been pursued as a new cancer therapeutic strategy. XIAP, cIAP1, and cIAP2, members of inhibitor of apoptosis (IAP) proteins, are critical regulators of cell death and survival and are attractive targets for new cancer therapy. The SMAC/DIABLO protein is an endogenous antagonist of XIAP, cIAP1, and cIAP2. In the last decade, intense research efforts have resulted in the design and development of several small-molecule SMAC mimetics now in clinical trials for cancer treatment

In a further preferred embodiment, the inventive composition comprises at least one RNA comprising at least one coding regaion that codes for at least one peptide or protein that binds inhibitors of apoptosis proteins (IAPs) and thus sensitize cancer cells to apoptotic death.

Therefore it is particularly preferred that the at least one RNA of the inventive RNA containing composition encodes at least one protein or peptide that bind inhibitors of apoptosis, such as SMAC mimetics.

Particularly preferred proteins or peptides that bind IAPs according to the present invention comprise Omi/HtrA2, Smac, Smac derived peptides, Smac/DIABLO, and XAF1 (XIAP-associated factor 1) and fragments or variants thereof.

RNA modifications

According to one embodiment, the at least one RNA of the composition, encoding at least one of the proteins and/or peptides defined herein, may be in the form of a modified RNA, wherein any modification, as defined herein, may be introduced into the at least one RNA of the composition. Modifications as defined herein preferably lead to a stabilization of the at least one RNA of the composition of the present invention.

According to one embodiment, the at least one RNA of the composition of the present invention may thus be provided as a “stabilized RNA", that is to say as an RNA 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 at least one RNA of the composition of 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 RNA 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 RNAs 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)).

In the following, specific modifications are described, which are preferably capable of "stabilizing" the at least one RNA as defined herein.

Chemical modifications:

The term "RNA modification" as used herein may refer to chemical modifications comprising backbone modifications as well as sugar modifications or base modifications.

In this context, a modified RNA as defined herein may contain nucleotide analogues/modifications, e.g. backbone modifications, sugar modifications or base modifications. A backbone modification in connection with the present invention is a modification, in which phosphates of the backbone of the nucleotides contained in an RNA as defined herein are chemically modified. A sugar modification in connection with the present invention is a chemical modification of the sugar of the nucleotides of the RNA as defined herein. Furthermore, a base modification in connection with the present invention is a chemical modification of the base moiety of the nucleotides of the RNA. In this context, nucleotide analogues or modifications are preferably selected from nucleotide analogues, which are applicable for transcription and/or translation.

Sugar Modifications:

The modified nucleosides and nucleotides, which may be incorporated into a modified RNA as described herein, can be modified in the sugar moiety. For example, the 2' hydroxyl group (OH) can be modified or replaced with a number of different "oxy" or "deoxy" substituents. Examples of "oxy" -2' hydroxyl group modifications include, but are not limited to, alkoxy or aryloxy (-OR, e.g., R = H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar); polyethyleneglycols (PEG), -$O(CH_2CH_2O)nCH_2CH_2OR$; "locked" nucleic acids (LNA) in which the 2' hydroxyl is connected, e.g., by a methylene bridge, to the 4' carbon of the same ribose sugar; and amino groups (-O-amino, wherein the amino group, e.g., NRR, can be alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroaryl amino, ethylene diamine, polyamino) or aminoalkoxy.

"Deoxy" modifications include hydrogen, amino (e.g. NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, diheteroaryl amino, or amino acid); or the amino group can be attached to the sugar through a linker, wherein the linker comprises one or more of the atoms C, N, and O.

The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified RNA can include nucleotides containing, for instance, arabinose as the sugar.

Backbone Modifications:

The phosphate backbone may further be modified in the modified nucleosides and nucleotides, which may be incorporated into a modified RNA as described herein. The phosphate groups of the backbone can be modified by replacing one or more of the oxygen atoms with a different substituent. Further, the modified nucleosides and nucleotides can include the full replacement of an unmodified phosphate moiety with a modified phosphate as described herein. Examples of modified phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulfur. The phosphate linker can also be modified by the replacement of a linking

oxygen with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylene-phosphonates).

Base Modifications:

The modified nucleosides and nucleotides, which may be incorporated into a modified RNA as described herein can further be modified in the nucleobase moiety. Examples of nucleobases found in RNA include, but are not limited to, adenine, guanine, cytosine and uracil. For example, the nucleosides and nucleotides described herein can be chemically modified on the major groove face. In some embodiments, the major groove chemical modifications can include an amino group, a thiol group, an alkyl group, or a halo group.

In particularly preferred embodiments of the present invention, the nucleotide analogues/modifications are selected from base modifications, which are preferably selected from 2-amino-6-chloropurineriboside-5'-triphosphate, 2-Aminopurine-riboside-5'-triphosphate; 2-aminoadenosine-5'-triphosphate, 2'-Amino-2'-deoxycytidine-triphosphate, 2-thiocytidine-5'-triphosphate, 2-thiouridine-5'-triphosphate, 2'-Fluorothymidine-5'-triphosphate, 2'-O-Methyl inosine-5'-triphosphate 4-thiouridine-5'-triphosphate, 5-aminoallylcytidine-5'-triphosphate, 5-aminoallyluridine-5'-triphosphate, 5-bromocytidine-5'-triphosphate, 5-bromouridine-5'-triphosphate, 5-Bromo-2'-deoxycytidine-5'-triphosphate, 5-Bromo-2'-deoxyuridine-5'-triphosphate, 5-iodocytidine-5'-triphosphate, 5-Iodo-2'-deoxycytidine-5'-triphosphate, 5-iodouridine-5'-triphosphate, 5-Iodo-2'-deoxyuridine-5'-triphosphate, 5-methylcytidine-5'-triphosphate, 5-methyluridine-5'-triphosphate, 5-Propynyl-2'-deoxycytidine-5'-triphosphate, 5-Propynyl-2'-deoxyuridine-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.

In some embodiments, modified nucleosides include pyridin-4-one ribonucleoside, 5-aza-uridine, 2-thio-5-aza-uridine, 2-thiouridine, 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxyuridine, 3-methyluridine, 5-carboxymethyl-uridine, 1-carboxymethyl-pseudouridine, 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyluridine, 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine, l-taurinomethyl-4-thio-uridine, 5-methyl-uridine, 1-methyl-pseudouridine, 4-thio- 1-methyl-pseudouridine, 2-thio-l-methyl-pseudouridine, 1 -methyl- 1-deaza-pseudouridine, 2-thio- 1 -methyl- 1 -deaza-pseudouridine, dihydrouridine, dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, and 4-methoxy-2-thio-pseudouridine.

In some embodiments, modified nucleosides include 5-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine, N4-acetylcytidine, 5-formylcytidine, N4-methylcytidine, 5-hydroxymethylcytidine, 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine, 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio- 1-methyl-pseudoisocytidine, 4-thio- 1 -methyl- 1 -deaza-pseudoisocytidine, 1 -methyl- 1 -deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, and 4-methoxy-l-methyl-pseudoisocytidine .

In other embodiments, modified nucleosides include 2-aminopurine, 2, 6-diaminopurine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-aminopurine, 7-deaza-8-aza-2-aminopurine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyladenosine, N6-methyladenosine, N6-isopentenyladenosine, N6-(cis-hydroxyisopentenyl)adenosine, 2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine, N6-glycinylcarbamoyladenosine, N6-threonylcarbamoyladenosine, 2-methylthio-N6-threonyl carbamoyladenosine, N6,N6-dimethyladenosine, 7-methyladenine, 2-methylthio-adenine, and 2-methoxy-adenine.

In other embodiments, modified nucleosides include inosine, 1-methyl-inosine, wyosine, wybutosine, 7-deaza-guanosine, 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine, 6-thio-7-methyl-guanosine, 7-methylinosine, 6-methoxy-guanosine, 1-methylguanosine, N2-methylguanosine, N2,N2-dimethylguanosine, 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, l-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, and N2,N2-dimethyl-6-thio-guanosine.

In some embodiments, the nucleotide can be modified on the major groove face and can include replacing hydrogen on C-5 of uracil with a methyl group or a halo group.

In specific embodiments, a modified nucleoside is 5'-O-(1-thiophosphate)-adenosine, 5'-O-(1-thiophosphate)-cytidine, 5'-O-(1-thiophosphate)-guanosine, 5'-O-(1-thiophosphate)-uridine or 5'-O-(1-thiophosphate)-pseudouridine.

In further specific embodiments, a modified RNA may comprise nucleoside modifications selected from 6-aza-cytidine, 2-thio-cytidine, α-thio-cytidine, Pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodo-uridine, N1-methyl-pseudouridine, 5,6-dihydrouridine, α-thio-uridine, 4-thio-uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, 5-methyl-uridine, Pyrrolo-cytidine, inosine, α-thio-guanosine, 6-methyl-guanosine, 5-methyl-cytdine, 8-oxo-guanosine, 7-deaza-guanosine, N1-methyl-adenosine, 2-amino-6-Chloro-purine, N6-methyl-2-amino-purine, Pseudo-iso-cytidine, 6-Chloro-purine, N6-methyl-adenosine, α-thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine.

Lipid modification:

According to a further embodiment, a modified RNA as defined herein can contain a lipid modification. Such a lipid-modified RNA typically comprises an RNA as defined herein. Such a lipid-modified RNA as defined herein typically further comprises at least one linker covalently linked with that RNA, and at least one lipid covalently linked with the respective linker. Alternatively, the lipid-modified RNA comprises at least one RNA as defined herein and at least one (bifunctional) lipid covalently linked (without a linker) with that RNA. According to a third alternative, the lipid-modified RNA comprises an RNA molecule as defined herein, at least one linker covalently linked with that RNA, 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 RNA. In this context, it is particularly preferred that the lipid modification is present at the terminal ends of a linear RNA sequence.

G/C content optimization:

According to an especially preferred embodiment of the invention, the RNA of the inventive composition is modified. Preferably the RNA is stabilized by modifying and preferably increasing the

G (guanosine)/C (cytosine) content of the RNA of the coding region thereof. Therein, the G/C content of the RNA of the coding region is increased compared to the G/C content of the coding region of its particular wild type coding sequence, i.e. the unmodified RNA. However, the encoded amino acid sequence of the RNA is preferably not modified compared to the encoded amino acid sequence of the particular wild type/unmodified RNA.

The modification of the G/C-content of the RNA of the inventive composition is based on the fact that RNA sequences having an increased G (guanosine)/C (cytosine) content are more stable than RNA sequences having an increased A (adenosine)/U (uracil) content. The codons of a coding sequence or a whole RNA might therefore be varied compared to the wild type coding sequence or RNA, such that they include an increased amount of G/C nucleotides while the translated amino acid sequence is retained. 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 favourable codons for the stability can be determined (so-called alternative codon usage). Depending on the amino acid to be encoded by the at least one RNA, there are various possibilities for modification of the RNA sequence, compared to its wild-type sequence. In the case of amino acids which are encoded by codons, which contain exclusively G or C nucleotides, no modification of the codon is necessary. Thus, the codons for Pro (CCC or CCG), Arg (CGC or CGG), Ala (GCC or GCG) and Gly (GGC or GGG) require no modification, since no A or U is present. In contrast, codons which contain A and/or U nucleotides can be 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 modified from CCU or CCA to CCC or CCG; the codons for Arg can be modified from CGU or CGA or AGA or AGG to CGC or CGG; the codons for Ala can be modified from GCU or GCA to GCC or GCG; the codons for Gly can be 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 modified from UUU to UUC; the codons for Leu can be modified from UUA, UUG, CUU or CUA to CUC or CUG; the codons for Ser can be modified from UCU or UCA or AGU to UCC, UCG or AGC; the codon for Tyr can be modified from UAU to UAC; the codon for Cys can be modified from UGU to UGC; the codon for His can be modified from CAU to CAC; the codon for Gln can be modified from CAA to CAG; the codons for Ile can be modified from AUU or AUA to AUC; the codons for Thr can be modified from ACU or ACA to ACC or ACG; the codon for Asn can be modified

from AAU to AAC; the codon for Lys can be modified from AAA to AAG; the codons for Val can be modified from GUU or GUA to GUC or GUG; the codon for Asp can be modified from GAU to GAC; the codon for Glu can be modified from GAA to GAG; the stop codon UAA can be 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 at least one mRNA of the composition of the present invention compared to its particular wild-type mRNA (i.e. the original sequence). Thus, for example, all codons for Thr occurring in the wild-type sequence can be 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 original sequence (wild-type mRNA) 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 at least one RNA according to the invention 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 wild type RNA. According to a specific embodiment at least 5%, 10%, 20%, 30%, 40%, 50%, 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 or peptide as defined herein or its fragment or variant thereof or the whole sequence of the wild type RNA sequence or coding sequence are substituted, thereby increasing the G/C content of said sequence. In this context, it is particularly preferable to increase the G/C content of the at least one RNA of the inventive composition to the maximum (i.e. 100% of the substitutable codons), in particular in the coding region, compared to the wild type sequence.

According to the invention, a further preferred modification of the coding sequence of the at least one RNA of the composition 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 the at least one coding region of the at least one RNA of the composition of the present invention to an increased extent, the corresponding modified at least one RNA sequence is translated to a significantly poorer degree than in the case where codons coding for relatively "frequent" tRNAs are present. According to the invention, in the modified at least one RNA of the composition of the present invention, the region which codes for one of the above defined peptides or proteins is modified compared to the corresponding region of the wild-type RNA such that at least one codon of the wild-type 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 sequence of the at least one coding region of the at least one RNA of the composition of the present invention is modified 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 wild-type 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 modified at least one RNA of the composition of the present invention, with the "frequent" codons without modifying the amino acid sequence of the protein encoded by the coding region of the RNA. This preferred embodiment allows provision of a particularly efficiently translated and stabilized (modified) at least one RNA of the composition of the present invention. The determination of a modified at least one RNA of the composition of the present invention as described above (increased G/C content; exchange of tRNAs) can be carried out using the computer program 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 RNA can be modified 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 modified at least one RNA preferably not being modified compared to the non-modified sequence. Alternatively, it is also possible to modify only the G/C content or only the codon usage compared to the original 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 the at least one RNA of the composition of the present invention is increased compared to the A/U content in the environment of the ribosome binding site of its particular wild-type RNA. This modification (an increased A/U content around the ribosome binding site) increases the efficiency of ribosome binding to the at least one RNA. An effective binding of the ribosomes to the ribosome binding site (Kozak sequence: GCCGCCACCAUGG (SEQ ID NO: 10.071), the AUG forms the start codon) in turn has the effect of an efficient translation of the at least one RNA. According to a further embodiment of the present invention the at least one RNA of the composition of the present invention may be modified with respect to potentially destabilizing sequence elements. Particularly, the coding region and/or the 5' and/or 3' untranslated region of this RNA may be modified compared to the particular wild-type RNA such that it contains no destabilizing sequence elements, the coded amino acid sequence of the modified at least one RNA preferably not being modified compared to its particular wild-type RNA. 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 modified at least one RNA, optionally in the region which encodes for a protein or peptide as defined herein, one or more such modifications compared to the corresponding region of the wild-type RNA can therefore be carried out, so that no or substantially no destabilizing sequence elements are contained there. According to the invention, DSE present in the untranslated regions (3'- and/or 5'-UTR) can also be eliminated from the at least one RNA of the composition of the present invention by such 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). The at least one RNA of the composition of the present invention is therefore preferably modified compared to the wild-type RNA such that the at least one RNA 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 in the at least one RNA of the composition of the present invention.

Adaptation to human codon usage:

According to the invention, a further preferred modification of the at least one RNA of the composition of the present invention is based on the finding that codons coding for the same amino acid occur in different frequencies. According to the invention, in the modified at least one RNA of the composition of the present invention, the region which codes for one of the above defined peptides or proteins (coding sequence) is preferably modified compared to the corresponding region of the wild-type RNA such that the frequency of the codons coding for the same amino acid corresponds to the naturally occurring frequency of that codon present in the human coding usage as e.g. shown in Table 13.

This means, for example, that for the amino acid Alanine (Ala) present in the amino acid sequence of the encoded protein according to the invention, the wild type coding sequence is adapted in a way that the codon “GCC” is used with a frequency of 0.40, the codon “GCT” is used with a frequency of 0.28, the codon “GCA” is used with a frequency of 0.22 and the codon “GCG” is used with a frequency of 0.10 etc. (see Table 13).

Table 13: Human codon usage table (most frequent codon marked with an asterisk)

Amino acid	codon	fraction	/1000
Ala	GCG	0.10	7.4
Ala	GCA	0.22	15.8
Ala	GCT	0.28	18.5
Ala	GCC*	0.40	27.7
Cys	TGT	0.42	10.6
Cys	TGC*	0.58	12.6
Asp	GAT	0.44	21.8
Asp	GAC*	0.56	25.1
Glu	GAG*	0.59	39.6
Glu	GAA	0.41	29.0
Phe	TTT	0.43	17.6
Phe	TTC*	0.57	20.3
Gly	GGG	0.23	16.5
Gly	GGA	0.26	16.5
Gly	GGT	0.18	10.8
Gly	GGC*	0.33	22.2
His	CAT	0.41	10.9
His	CAC*	0.59	15.1
Ile	ATA	0.14	7.5
Ile	ATT	0.35	16.0
Ile	ATC*	0.52	20.8
Lys	AAG*	0.60	31.9
Lys	AAA	0.40	24.4
Leu	TTG	0.12	12.9
Leu	TTA	0.06	7.7
Leu	CTG*	0.43	39.6
Leu	CTA	0.07	7.2

Amino acid	codon	fraction	/1000
Leu	CTT	0.12	13.2
Leu	CTC	0.20	19.6
Met	ATG*	1	22.0
Asn	AAT	0.44	17.0
Asn	AAC*	0.56	19.1
Pro	CCG	0.11	6.9
Pro	CCA	0.27	16.9
Pro	CCT	0.29	17.5
Pro	CCC*	0.33	19.8
Gln	CAG*	0.73	34.2
Gln	CAA	0.27	12.3
Arg	AGG	0.22	12.0
Arg	AGA*	0.21	12.1
Arg	CGG	0.19	11.4
Arg	CGA	0.10	6.2
Arg	CGT	0.09	4.5
Arg	CGC	0.19	10.4
Ser	AGT	0.14	12.1
Ser	AGC*	0.25	19.5
Ser	TCG	0.06	4.4
Ser	TCA	0.15	12.2
Ser	TCT	0.18	15.2
Ser	TCC	0.23	17.7
Thr	ACG	0.12	6.1
Thr	ACA	0.27	15.1
Thr	ACT	0.23	13.1
Thr	ACC*	0.38	18.9
Val	GTG*	0.48	28.1
Val	GTA	0.10	7.1

Amino acid	codon	fraction	/1000
Val	GTT	0.17	11.0
Val	GTC	0.25	14.5
Trp	TGG*	1	13.2
Tyr	TAT	0.42	12.2
Tyr	TAC*	0.58	15.3
Stop	TGA*	0.61	1.6
Stop	TAG	0.17	0.8
Stop	TAA	0.22	1.0

Codon-optimization:

According to a particularly preferred embodiment it is preferred, that all codons of the wild-type sequence of the coding region of the at least one RNA of the inventive composition which code for a tRNA which is relatively rare in the cell is in each case 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. Therefore it is particularly preferred that the most frequent codons are used for each encoded amino acid (see Table 13, most frequent codons are marked with asterisks).

This means, for example, that for the amino acid Alanine (Ala) present in the amino acid sequence of the encoded peptide or protein according to the invention, the wild type coding sequence is adapted in a way that the most frequent human codon "GCC" is always used for said amino acid, or for the amino acid Cysteine (Cys), the wild type sequence is adapted in a way that the most frequent human codon "TGC" is always used for said amino acid etc.

C-enrichment:

According to another embodiment, the at least one RNA of the composition of the present invention may be modified by increasing the C content of the RNA, preferably of the coding region of the at least one RNA.

In a particularly preferred embodiment of the present invention, the C content of the coding region of the at least one RNA of the composition of the present invention is modified, particularly

increased, compared to the C content of the coding region of its particular wild-type RNA, i.e. the unmodified mRNA. The amino acid sequence encoded by the at least one RNA is preferably not modified as compared to the amino acid sequence encoded by the particular wild-type RNA

In a preferred embodiment of the present invention, the modified RNA is modified such that at least 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80%, or at least 90% of the theoretically maximal cytosine-content or even a maximal cytosine-content is achieved.

In further preferred embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or even 100% of the codons of the target RNA wild type sequence, which are "cytosine content optimizable" are replaced by codons with a higher cytosine-content as present in the wild type sequence.

In a further preferred embodiment, some of the codons of the wild type coding sequence may additionally be modified such that a codon for a relatively rare tRNA in the cell is exchanged by a codon for a relatively frequent tRNA in the cell, provided that the substituted codon for a relatively frequent tRNA carries the same amino acid as the relatively rare tRNA of the original wild type codon. Preferably, all of the codons for a relatively rare tRNA are replaced by a codon for a relatively frequent tRNA in the cell, except codons encoding amino acids, which are exclusively encoded by codons not containing any cytosine, or except for glutamine (Gln), which is encoded by two codons each containing the same number of cytosines.

In a further preferred embodiment of the present invention, the modified target RNA is modified such that at least 80%, or at least 90% of the theoretically maximal cytosine-content or even a maximal cytosine-content is achieved by means of codons, which code for relatively frequent tRNAs in the cell, wherein the amino acid sequence remains unchanged.

Due to the naturally occurring degeneracy of the genetic code, more than one codon may encode a particular amino acid. Accordingly, 18 out of 20 naturally occurring amino acids are encoded by more than 1 codon (with Tryp and Met being an exception), e.g. by 2 codons (e.g. Cys, Asp, Glu), by three codons (e.g. Ile), by 4 codons (e.g. Al, Gly, Pro) or by 6 codons (e.g. Leu, Arg, Ser). However, not all codons encoding the same amino acid are utilized equally frequent under in vivo conditions. Depending on each single organism, a typical codon usage profile is established.

The term "cytosine content-optimizable codon" as used within the context of the present invention refers to codons, which exhibit a lower amount of cytosines than other codons coding for the same amino acid. Accordingly, any wild type codon, which may be replaced by another codon coding for the same amino acid and exhibiting a higher number of cytosines within that codon, is considered to be cytosine-optimizable (C-optimizable). Any such substitution of a C-optimizable wild type codon by the specific C-optimized codon within a wild type coding region increases its overall C-content and reflects a C-enriched modified RNA sequence. A C-maximized RNA sequence contains C-optimized codons for all potentially C-optimizable codons. Accordingly, 100% or all of the theoretically replaceable C-optimizable codons are under such conditions actually replaced by C-optimized codons over the entire length of the coding region.

In this context, cytosine-content optimizable codons are codons, which contain a lower number of cytosines than other codons coding for the same amino acid.

Any of the codons GCG, GCA, GCU codes for the amino acid Ala, which may be exchanged by the codon GCC encoding the same amino acid, and/or

the codon UGU that codes for Cys may be exchanged by the codon UGC encoding the same amino acid, and/or

the codon GAU which codes for Asp may be exchanged by the codon GAC encoding the same amino acid, and/or

the codon that UUU that codes for Phe may be exchanged for the codon UUC encoding the same amino acid, and/or

any of the codons GGG, GGA, GGU that code Gly may be exchanged by the codon GGC encoding the same amino acid, and/or

the codon CAU that codes for His may be exchanged by the codon CAC encoding the same amino acid, and/or

any of the codons AUA, AUU that code for Ile may be exchanged by the codon AUC, and/or

any of the codons UUG, UUA, CUG, CUA, CUU coding for Leu may be exchanged by the codon CUC encoding the same amino acid, and/or

the codon AAU that codes for Asn may be exchanged by the codon AAC encoding the same amino acid, and/or

any of the codons CCG, CCA, CCU coding for Pro may be exchanged by the codon CCC encoding the same amino acid, and/or

any of the codons AGG, AGA, CGG, CGA, CGU coding for Arg may be exchanged by the codon CGC encoding the same amino acid, and/or

any of the codons AGU, AGC, UCG, UCA, UCU coding for Ser may be exchanged by the codon UCC encoding the same amino acid, and/or

any of the codons ACG, ACA, ACU coding for Thr may be exchanged by the codon ACC encoding the same amino acid, and/or

any of the codons GUG, GUA, GUU coding for Val may be exchanged by the codon GUC encoding the same amino acid, and/or

the codon UAU coding for Tyr may be exchanged by the codon UAC encoding the same amino acid.

In any of the above instances, the number of cytosines is increased by 1 per exchanged codon. Exchange of all non C-optimized codons (corresponding to C-optimizable codons) of the coding region results in a C-maximized coding sequence. In the context of the invention at least 70% of the non C-optimized codons are replaced by C-optimized codons of the wild type sequence are replaced by C-optimized codons, preferably at least 80%, more preferably at least 90% within the coding region.

It may be preferred that for some amino acids the percentage of C-optimizable codons replaced by C-optimized codons is less than 70%, while for other amino acids the percentage of replaced codons is higher than 70% to meet the overall percentage of C-optimization of at least 70% of all C-optimizable wild type codons of the coding region.

Preferably, in the C-optimized RNAs of the invention, at least 50% of the C-optimizable wild type codons for any given amino acid are replaced by C-optimized codons, e.g. any modified C-enriched RNA preferably contains at least 50% C-optimized codons at C-optimizable wild type codon positions coding for any single of the above mentioned amino acids Ala, Cys, Asp, Phe, Gly, His, Ile, Leu, Asn, Pro, Arg, Ser, Thr, Val and Tyr, preferably at least 60%.

In this context codons coding for amino acids, which are not cytosine content-optimizable and which are, however, encoded by at least two codons, may be used without any further selection process. However, the codon of the wild type sequence that codes for a relatively rare tRNA in the cell, e.g. a human cell, may be exchanged for a codon that codes for a relatively frequent tRNA in

the cell, whereby both code for the same amino acid. Accordingly, the relatively rare codon GAA coding for Glu may be exchanged by the relative frequent codon GAG coding for the same amino acid, and/or

the relatively rare codon AAA coding for Lys may be exchanged by the relative frequent codon AAG coding for the same amino acid, and/or

the relatively rare codon CAA coding for Gln is exchanged for the relative frequent codon CAG encoding the same amino acid.

In this context, the amino acids Met (AUG) and Trp (UGG), which are encoded by only one codon each, remain unchanged. Stop codons are not cytosine-content optimized, however, the relatively rare stop codons amber, ochre (UAA, UAG) may be exchanged by the relatively frequent stop codon opal (UGA).

The substitutions listed above may obviously be used individually but also in all possible combinations in order to optimize the cytosine-content of the modified RNA compared to the wild type RNA sequence.

Accordingly, the region of the modified RNA coding for the peptide or protein may be changed compared to the coding region of the wild type RNA in such a way that an amino acid encoded by at least two or more codons, of which one comprises one additional cytosine, such a codon may be exchanged by the C-optimized codon comprising one additional cytosine, whereby the amino acid is unaltered compared to the wild type sequence.

Substitutions, additions or eliminations of bases are preferably carried out using a DNA matrix for preparation of the nucleic acid molecule by techniques of the well known site directed mutagenesis or with an oligonucleotide ligation. In such a process, for preparation of the at least one RNA as defined herein a corresponding DNA molecule may be transcribed *in vitro*. This DNA matrix preferably comprises a suitable promoter, e.g. a T7 or SP6 promoter, for *in vitro* transcription, which is followed by the desired nucleotide sequence for the at least one RNA to be prepared and a termination signal for *in vitro* transcription. The DNA molecule, which forms the matrix of the at least one RNA of interest, may be prepared by fermentative proliferation and subsequent isolation as part of a plasmid which can be replicated in bacteria. Plasmids which may be mentioned as

suitable for the present invention are e.g. the plasmids pT7Ts (GenBank accession number U26404; Lai *et al.*, Development 1995, 121: 2349 to 2360), pGEM® series, e.g. pGEM®-1 (GenBank accession number X65300; from Promega) and pSP64 (GenBank accession number X65327); cf. also Mezei and Storts, Purification of PCR Products, in: Griffin and Griffin (ed.), PCR Technology: Current Innovation, CRC Press, Boca Raton, FL, 2001.

Fragments and variants

In the context of the invention additionally to the here disclosed peptides and proteins, which show a certain degree of identity of sequence, are incorporated. Therefore fragments and variants of the proteins and peptides as defineded herein are disclosed herewith in the context of the present invention.

Furthermore fragments and variants of nucleic acids as defined herein are therefore disclosed herewith in the context of the present invention.

Mono-Bi-Multicistronic, Self cleaving peptides etc:

The coding region of the at least oneRNA of the inventive composition may occur as a mono-, di-, or even multicistronic RNA, i.e. an RNA sequence which carries the coding sequences of one, two or more proteins or peptides. Such coding sequences of the di-, or even multicistronic RNAs may be separated by at least one internal ribosome entry site (IRES) sequence. Thus, the at least one RNA according to the invention may further comprise one or more internal ribosome entry site (IRES) sequences or IRES-motifs, which may separate several open reading frames, especially if the RNA encodes for two or more peptides or proteins (bi- or multicistronic RNA). For example, the internal ribosome entry site sequence may be derived from EMCV (encephalomyocarditis virus) or from FMDV (Foot and mouth disease virus). Furthermore self-cleaving signal peptides may be used which induce the cleavage of the resulting polypeptide which comprises several proteins or peptides, e.g. a self-cleaving signal peptide sequence derived from F2A peptide from FMDV.

Combinations of different coding sequences

In a preferred embodiment, the inventive composition comprises at least one, two, three, four, five, six, seven, eight, nine, ten or more RNAs, each comprising at least one, two, three, four, five, six, seven, eight, nine, ten or more coding regions encoding at least one or more cytokine as

defined above and/or at least one or more chemokine as defined above, and/or at least one or more suicide gene product as definded above, and/or at least one or more immunogenic peptide or protein as defined above, and/or at least one or more apoptosis inducer as defined above, and/or at least one or more angiogenesis inhibitor as defined above, and/or at least one or more heat shock protein as defined above, and/or at least one or more tumor antigen as defined above, and/or at least one or more β-catenin inhibitor as defined above, and/or at least one or more STING pathway activator as defined above, and/or at least one or more checkpoint modulator as defined above, and/or at least one or more innate immune activator, and/or at least one or more antibody as defined above, and/or at least one dominant negative receptor and/or at least one or more decoy receptor, and/or at least one or more inhibitor of myeloid derived suppressor cells (MDSCs), and/or at least one or more IDO pathway inhibitor, and/or at least one or more protein or peptide that bind apoptosis inhibitors as defined above, or variants orfragments thereof.

Untranslated regions (UTRs)

By a further embodiment the at least one RNA of the inventive composition preferably comprises at least one of the following structural elements: a 5'- and/or 3'- untranslated region element (UTR element), particularly a 5'-UTR element which comprises or consists of a nucleic acid sequence which is derived from the 5'-UTR of a TOP gene or from a fragment, homolog or a variant thereof, or a 5'- and/or 3'-UTR element which may be derivable from a gene that provides a stable mRNA or from a homolog, fragment or variant thereof; a histone stem-loop structure, preferably a histone stem-loop in its 3' untranslated region; a 5'-CAP structure; a poly-A tail (poly(A) sequence); or a poly(C) sequence.

In a preferred embodiment the at least one RNA comprises at least one 5'- or 3'-UTR element. In this context an UTR element comprises or consists of a nucleic acid sequence which is derived from the 5'- or 3'-UTR of any naturally occurring gene or which is derived from a fragment, a homolog or a variant of the 5'- or 3'-UTR of a gene. Preferably the 5'- or 3'-UTR element used according to the present invention is heterologous to the coding region of the RNA of the inventive composition. Even if 5'- or 3'-UTR elements derived from naturally occurring genes are preferred, also synthetically engineered UTR elements may be used in the context of the present invention.

In a particularly preferred embodiment the at least one RNA comprises at least one 5'-untranslated region element (5'-UTR element) which comprises or consists of a nucleic acid sequence which is derived from the 5'-UTR of a TOP gene or which is derived from a fragment, homolog or variant of the 5'-UTR of a TOP gene.

It is particularly preferred that the 5'-UTR element does not comprise a TOP-motif or a 5'-TOP, as defined above.

In some embodiments, the nucleic acid sequence of the 5'-UTR element which is derived from a 5'-UTR of a TOP gene terminates at its 3'-end with a nucleotide located at position 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 upstream of the start codon (e.g. A(U/T)G) of the gene or mRNA it is derived from. Thus, the 5'-UTR element does not comprise any part of the protein coding region. Thus, preferably, the only protein coding part of mRNA of the inventive composition is provided by the coding region.

The nucleic acid sequence which is derived from the 5'-UTR of a TOP gene is preferably derived from a eukaryotic TOP gene, preferably a plant or animal TOP gene, more preferably a chordate TOP gene, even more preferably a vertebrate TOP gene, most preferably a mammalian TOP gene, such as a human TOP gene.

For example, the 5'-UTR element is preferably selected from 5'-UTR elements comprising or consisting of a nucleic acid sequence which is derived from a nucleic acid sequence selected from the group consisting of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, whose disclosure is incorporated herein by reference, from the homologs of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, from a variant thereof, or preferably from a corresponding RNA sequence. The term "homologs of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700" refers to sequences of other species than homo sapiens, which are homologous to the sequences according to SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700.

In a preferred embodiment, the 5'-UTR element comprises or consists of a nucleic acid sequence which is derived from a nucleic acid sequence extending from nucleotide position 5 (i.e. the nucleotide that is located at position 5 in the sequence) to the nucleotide position immediately 5' to the start codon (located at the 3' end of the sequences), e.g. the nucleotide position immediately 5' to the ATG sequence, of a nucleic acid sequence selected from SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, from the homologs of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700 from a variant thereof, or a corresponding RNA sequence. It is particularly preferred that the 5'-UTR element is derived from a nucleic acid sequence extending from the nucleotide position immediately 3' to the 5'-TOP to the nucleotide position immediately 5' to the start codon (located at the 3' end of the sequences), e.g. the nucleotide position immediately 5' to the ATG sequence, of a nucleic acid sequence selected from SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, from the homologs of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, from a variant thereof, or a corresponding RNA sequence.

In a particularly preferred embodiment, the 5'-UTR element comprises or consists of a nucleic acid sequence which is derived from a 5'-UTR of a TOP gene encoding a ribosomal protein or from a variant of a 5'-UTR of a TOP gene encoding a ribosomal protein. For example, the 5'-UTR element comprises or consists of a nucleic acid sequence which is derived from a 5'-UTR of a nucleic acid sequence according to any of SEQ ID NOs: 67, 170, 193, 244, 259, 554, 650, 675, 700, 721, 913, 1016, 1063, 1120, 1138, and 1284-1360 of the patent application WO2013/143700, a corresponding RNA sequence, a homolog thereof, or a variant thereof as described herein, preferably lacking the 5'-TOP motif. As described above, the sequence extending from position 5 to the nucleotide immediately 5' to the ATG (which is located at the 3'end of the sequences) corresponds to the 5'-UTR of said sequences.

Preferably, the 5'-UTR element comprises or consists of a nucleic acid sequence which is derived from a 5'-UTR of a TOP gene encoding a ribosomal large protein (RPL) or from a homolog or variant of a 5'-UTR of a TOP gene encoding a ribosomal large protein (RPL). For example, the 5'-UTR element comprises or consists of a nucleic acid sequence which is derived from a 5'-UTR of a

nucleic acid sequence according to any of SEQ ID NOs: 67, 259, 1284-1318, 1344, 1346, 1348-1354, 1357, 1358, 1421 and 1422 of the patent application WO2013/143700, a corresponding RNA sequence, a homolog thereof, or a variant thereof as described herein, preferably lacking the 5'-TOP motif.

In a particularly preferred embodiment, the 5'-UTR element comprises or consists of a nucleic acid sequence which is derived from the 5'-UTR of a ribosomal protein Large 32 gene, preferably from a vertebrate ribosomal protein Large 32 (L32) gene, more preferably from a mammalian ribosomal protein Large 32 (L32) gene, most preferably from a human ribosomal protein Large 32 (L32) gene, or from a variant of the 5'-UTR of a ribosomal protein Large 32 gene, preferably from a vertebrate ribosomal protein Large 32 (L32) gene, more preferably from a mammalian ribosomal protein Large 32 (L32) gene, most preferably from a human ribosomal protein Large 32 (L32) gene, wherein preferably the 5'-UTR element does not comprise the 5'-TOP of said gene.

A preferred sequence for a 5'-UTR element corresponds to SEQ ID No. 1368 of the patent application WO2013/143700.

Accordingly, in a particularly preferred embodiment, the 5'-UTR element comprises or consists of a nucleic acid sequence which has an identity of at least about 20%, preferably of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence as mentioned above (according to SEQ ID NO. 10.051 (5'-UTR of human ribosomal protein Large 32 lacking the 5' terminal oligopyrimidine tract: GGCGCTGCCTACGGAGGTGGCAGCCATCTCCTTCTCGGCATC; corresponding to SEQ ID No. 1368 of the patent application WO2013/143700)) or preferably to a corresponding RNA sequence, or wherein the at least one 5'UTR element comprises or consists of a fragment of a nucleic acid sequence which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NO. 10.052 or more preferably to a corresponding RNA sequence, wherein, preferably, the fragment is as described

above, i.e. being a continuous stretch of nucleotides representing at least 20% etc. of the full-length 5'-UTR.

Preferably, the fragment exhibits a length of at least about 20 nucleotides or more, preferably of at least about 30 nucleotides or more, more preferably of at least about 40 nucleotides or more. Preferably, the fragment is a functional fragment as described herein.

In some embodiments, the mRNA of the inventive composition comprises a 5'-UTR element which comprises or consists of a nucleic acid sequence which is derived from the 5'-UTR of a vertebrate TOP gene, such as a mammalian, e.g. a human TOP gene, selected from RPSA, RPS2, RPS3, RPS3A, RPS4, RPS5, RPS6, RPS7, RPS8, RPS9, RPS10, RPS11, RPS12, RPS13, RPS14, RPS15, RPS15A, RPS16, RPS17, RPS18, RPS19, RPS20, RPS21, RPS23, RPS24, RPS25, RPS26, RPS27, RPS27A, RPS28, RPS29, RPS30, RPL3, RPL4, RPL5, RPL6, RPL7, RPL7A, RPL8, RPL9, RPL10, RPL10A, RPL11, RPL12, RPL13, RPL13A, RPL14, RPL15, RPL17, RPL18, RPL18A, RPL19, RPL21, RPL22, RPL23, RPL23A, RPL24, RPL26, RPL27, RPL27A, RPL28, RPL29, RPL30, RPL31, RPL32, RPL34, RPL35, RPL35A, RPL36, RPL36A, RPL37, RPL37A, RPL38, RPL39, RPL40, RPL41, RPLP0, RPLP1, RPLP2, RPLP3, RPLP0, RPLP1, RPLP2, EEF1A1, EEF1B2, EEF1D, EEF1G, EEF2, EIF3E, EIF3F, EIF3H, EIF2S3, EIF3C, EIF3K, EIF3EIP, EIF4A2, PABPC1, HNRNPA1, TPT1, TUBB1, UBA52, NPM1, ATP5G2, GNB2L1, NME2, UQCRB, or from a homolog or variant thereof, wherein preferably the 5'-UTR element does not comprise a TOP-motif or the 5'-TOP of said genes, and wherein optionally the 5'-UTR element starts at its 5'-end with a nucleotide located at position 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 downstream of the 5' terminal oligopyrimidine tract (TOP) and wherein further optionally the 5'-UTR element which is derived from a 5'-UTR of a TOP gene terminates at its 3'-end with a nucleotide located at position 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 upstream of the start codon (A(U/T)G) of the gene it is derived from.

In further particularly preferred embodiments, the 5'-UTR element comprises or consists of a nucleic acid sequence which is derived from the 5'-UTR of a ribosomal protein Large 32 gene (RPL32), a ribosomal protein Large 35 gene (RPL35), a ribosomal protein Large 21 gene (RPL21), an ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1) gene, an hydroxysteroid (17-beta) dehydrogenase 4 gene (HSD17B4), an androgen-induced 1 gene (AIG1), cytochrome c oxidase subunit VIc gene (COX6C), or a N-acylsphingosine amidohydrolase (acid ceramidase) 1 gene (ASAH1) or from a variant thereof, preferably from a vertebrate ribosomal

protein Large 32 gene (RPL32), a vertebrate ribosomal protein Large 35 gene (RPL35), a vertebrate ribosomal protein Large 21 gene (RPL21), a vertebrate ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1) gene, a vertebrate hydroxysteroid (17-beta) dehydrogenase 4 gene (HSD17B4), a vertebrate androgen-induced 1 gene (AIG1), a vertebrate cytochrome c oxidase subunit VIc gene (COX6C), or a vertebrate N-acylsphingosine amidohydrolase (acid ceramidase) 1 gene (ASAH1) or from a variant thereof, more preferably from a mammalian ribosomal protein Large 32 gene (RPL32), a ribosomal protein Large 35 gene (RPL35), a ribosomal protein Large 21 gene (RPL21), a mammalian ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1) gene, a mammalian hydroxysteroid (17-beta) dehydrogenase 4 gene (HSD17B4), a mammalian androgen-induced 1 gene (AIG1), a mammalian cyto-chrome c oxidase subunit VIc gene (COX6C), or a mammalian N-acylsphingosine ami-dohydrolase (acid ceramidase) 1 gene (ASAH1) or from a variant thereof, most preferably from a human ribosomal protein Large 32 gene (RPL32), a human ribosomal protein Large 35 gene (RPL35), a human ribosomal protein Large 21 gene (RPL21), a human ATP syn-thase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1) gene, a human hydroxysteroid (17-beta) dehydrogenase 4 gene (HSD17B4), a human androgen-induced 1 gene (AIG1), a human cytochrome c oxidase subunit VIc gene (COX6C), or a human N-acylsphingosine amidohydrolase (acid ceramidase) 1 gene (ASAH1) or from a variant thereof, wherein preferably the 5'-UTR element does not comprise the 5'-TOP of said gene.
In this context particularly preferred are 5'-UTR elements comprising a nucleic acid sequence according to SEQ ID Nos. 10.051-10.054.

Accordingly, in a particularly preferred embodiment, the 5'-UTR element comprises or consists of a nucleic acid sequence which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID No. 1368, or SEQ ID NOs 1412-1420 of the patent application WO2013/143700, or a corresponding RNA sequence, or wherein the at least one 5'-UTR element comprises or consists of a fragment of a nucleic acid sequence which has an identity of at least about 20%, preferably of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more

preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID No. 1368, or SEQ ID NOs 1412-1420 of the patent application WO2013/143700, wherein, preferably, the fragment is as described above, i.e. being a continuous stretch of nucleotides representing at least 20% etc. of the full-length 5'-UTR. Preferably, the fragment exhibits a length of at least about 20 nucleotides or more, preferably of at least about 30 nucleotides or more, more preferably of at least about 40 nucleotides or more. Preferably, the fragment is a functional fragment as described herein.

Accordingly, in a particularly preferred embodiment, the 5'-UTR element comprises or consists of a nucleic acid sequence which has an identity of at least about 20%, preferably of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID No. 10.053 (5'-UTR of ATP5A1 lacking the 5' terminal oligopyrimidine tract:
GCGGCTCGGCCATTTTGTCCCAGTCAGTCCGGAGGCTGCGGCTGCAGAAGTACCGCCTGCG-
GAGTAACTGCAAAG; corresponding to SEQ ID No. 1414 of the patent application WO2013/143700 (5'-UTR of ATP5A1 lacking the 5' terminal oligopyrimidine tract) or preferably to a corresponding RNA sequence, or wherein the at least one 5'UTR element comprises or consists of a fragment of a nucleic acid sequence which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NO. 26 (of the patent application WO2013/143700) or more preferably to a corresponding RNA sequence, wherein, preferably, the fragment is as described above, i.e. being a continuous stretch of nucleotides representing at least 20% etc. of the full-length 5'-UTR. Preferably, the fragment exhibits a length of at least about 20 nucleotides or more, preferably of at least about 30 nucleotides or more, more preferably of at least about 40 nucleotides or more. Preferably, the fragment is a functional fragment as described herein.

In a further preferred embodiment, the at least one RNA of the inventive composition further comprises at least one 3'-UTR element which comprises or consists of a nucleic acid sequence

derived from the 3'-UTR of a chordate gene, preferably a vertebrate gene, more preferably a mammalian gene, most preferably a human gene, or from a variant of the 3'-UTR of a chordate gene, preferably a vertebrate gene, more preferably a mammalian gene, most preferably a human gene.

The term '3'-UTR element' refers to a nucleic acid sequence which comprises or consists of a nucleic acid sequence that is derived from a 3'-UTR or from a variant of a 3'-UTR. A 3'-UTR element in the sense of the present invention may represent the 3'-UTR of an mRNA. Thus, in the sense of the present invention, preferably, a 3'-UTR element may be the 3'-UTR of an mRNA, preferably of an artificial mRNA, or it may be the transcription template for a 3'-UTR of an mRNA. Thus, a 3'-UTR element preferably is a nucleic acid sequence which corresponds to the 3'-UTR of an mRNA, preferably to the 3'-UTR of an artificial mRNA, such as an mRNA obtained by transcription of a genetically engineered vector construct. Preferably, the 3'-UTR element fulfils the function of a 3'-UTR or encodes a sequence which fulfils the function of a 3'-UTR.

Preferably, the inventive mRNA comprises a 3'-UTR element which may be derivable from a gene that relates to an mRNA with an enhanced half-life (that provides a stable mRNA), for example a 3'-UTR element as defined and described below. Preferably, the 3'-UTR element, is a nucleic acid sequence derived from a 3'-UTR of a gene, which preferably encodes a stable mRNA, or from a homolog, a fragment or a variant of said gene

In a particularly preferred embodiment, the 3'-UTR element comprises or consists of a nucleic acid sequence which is derived from a 3'-UTR of a gene selected from the group consisting of an albumin gene, an α-globin gene, a β-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, and a collagen alpha gene, such as a collagen alpha 1(I) gene, or from a variant of a 3'-UTR of a gene selected from the group consisting of an albumin gene, an α-globin gene, a β-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, and a collagen alpha gene, such as a collagen alpha 1(I) gene according to SEQ ID No. 1369-1390 of the patent application WO2013/143700 whose disclosure is incorporated herein by reference. In a particularly preferred embodiment, the 3'-UTR element comprises or consists of a nucleic acid sequence which is derived from a 3'-UTR of an albumin gene, preferably a vertebrate albumin gene, more preferably a mammalian albumin gene,

most preferably a human albumin gene, most preferably a human albumin gene according to SEQ ID NO. 10063 (according SEQ ID No: 1369 of the patent application WO2013/143700). The mRNA sequence may comprise or consist of a nucleic acid sequence which is derived from the 3'-UTR of the human albumin gene according to GenBank Accession number NM_000477.5, or from a fragment or variant thereof.

In this context it is particularly preferred that the mRNA of the inventive composition comprises a 3'-UTR element comprising a corresponding RNA sequence derived from the nucleic acids according to SEQ ID No. 1369-1390 of the patent application WO2013/143700 or a fragment, homolog or variant thereof.

Most preferably the 3'-UTR element comprises the nucleic acid sequence derived from a fragment of the human albumin gene (albumin7 3'UTR) according to SEQ ID NO. 10065 (according to SEQ ID No: 1376 of the patent application WO2013/143700).

In this context it is particularly preferred that the 3'-UTR element of the at least one RNA of the inventive composition comprises or consists of a corresponding RNA sequence of the nucleic acid sequence according to SEQ ID NO. 10066.

In another particularly preferred embodiment, the 3'-UTR element comprises or consists of a nucleic acid sequence which is derived from a 3'-UTR of an α-globin gene, preferably a vertebrate α- or β-globin gene, more preferably a mammalian α- or β-globin gene, most preferably a human α- or β-globin gene according to SEQ ID NO. 10055 (corresponding to SEQ ID No. 1370 of the patent application WO2013/143700 (3'-UTR of Homo sapiens hemoglobin, alpha 1 (HBA1))), or according to SEQ ID NO. 10057 (corresponding to SEQ ID No. 1371 of the patent application WO2013/143700 (3'-UTR of Homo sapiens hemoglobin, alpha 2 (HBA2))), and/or according to SEQ ID NO. 10059 (corresponding to SEQ ID No. 1372 of the patent application WO2013/143700 (3'-UTR of Homo sapiens hemoglobin, beta (HBB)).

For example, the 3'-UTR element may comprise or consist of the center, α-complex-binding portion of the 3'-UTR of an α-globin gene, according to SEQ ID NO. 10061 (corresponding to SEQ ID No. 1393 of the patent application WO2013/143700).

In this context it is particularly preferred that the 3'-UTR element of the RNA of the inventive composition comprises or consists of a corresponding RNA sequence of the nucleic acid sequence according to SEQ ID NO. 10062, according to the above or a homolog, a fragment or variant thereof.

The term 'a nucleic acid sequence which is derived from the 3'-UTR of a [...] gene' preferably refers to a nucleic acid sequence which is based on the 3'-UTR sequence of a [...] gene or on a part thereof, such as on the 3'-UTR of an albumin gene, an α-globin gene, a β-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, or a collagen alpha gene, such as a collagen alpha 1(I) gene, preferably of an albumin gene or on a part thereof. This term includes sequences corresponding to the entire 3'-UTR sequence, i.e. the full length 3'-UTR sequence of a gene, and sequences corresponding to a fragment of the 3'-UTR sequence of a gene, such as an albumin gene, α-globin gene, β-globin gene, tyrosine hydroxylase gene, lipoxygenase gene, or collagen alpha gene, such as a collagen alpha 1(I) gene, preferably of an albumin gene.

The term 'a nucleic acid sequence which is derived from a variant of the 3'-UTR of a [...] gene' preferably refers to a nucleic acid sequence which is based on a variant of the 3'-UTR sequence of a gene, such as on a variant of the 3'-UTR of an albumin gene, an α-globin gene, a β-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, or a collagen alpha gene, such as a collagen alpha 1(I) gene, or on a part thereof as described above. This term includes sequences corresponding to the entire sequence of the variant of the 3'-UTR of a gene, i.e. the full length variant 3'-UTR sequence of a gene, and sequences corresponding to a fragment of the variant 3'-UTR sequence of a gene. A fragment in this context preferably consists of a continuous stretch of nucleotides corresponding to a continuous stretch of nucleotides in the full-length variant 3'-UTR, which represents at least 20%, preferably at least 30%, more preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, even more preferably at least 70%, even more preferably at least 80%, and most preferably at least 90% of the full-length variant 3'-UTR. Such a

fragment of a variant, in the sense of the present invention, is preferably a functional fragment of a variant as described herein.

Preferably, the at least one 5'-UTR element and the at least one 3'-UTR element act synergistically to increase protein production from the RNA of the inventive composition as described above.

Histone stem loop:

In a particularly preferred embodiment, the at least oneRNA of the inventive composition comprises a histone stem-loop sequence/structure. Such histone stem-loop sequences are preferably selected from histone stem-loop sequences as disclosed in WO 2012/019780, whose disclosure is incorporated herewith by reference.

A histone stem-loop sequence, suitable to be used within the present invention, is preferably selected from at least one of the following formulae (I) or (II):

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

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

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

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

bordering element — bordering element

wherein:

stem1 or stem2 bordering elements N_{1-6}	is a consecutive sequence of 1 to 6, preferably of 2 to 6, more preferably of 2 to 5, even more preferably of 3 to 5, most preferably of 4 to 5 or 5 N, wherein each

	N is independently from another selected from a nucleotide selected from A, U, T, G and C, or a nucleotide analogue thereof;
stem1 [$N_{0-2}GN_{3-5}$]	is reverse complementary or partially reverse complementary with element stem2, and is a consecutive sequence between of 5 to 7 nucleotides;

wherein N_{0-2} is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

wherein N_{3-5} is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof, and

wherein G is guanosine or an analogue thereof, and may be optionally replaced by a cytidine or an analogue thereof, provided that its complementary nucleotide cytidine in stem2 is replaced by guanosine;

loop sequence [$N_{0-4}(U/T)N_{0-4}$]	is located between elements stem1 and stem2, and is a consecutive sequence of 3 to 5 nucleotides, more preferably of 4 nucleotides;

wherein each N_{0-4} is independent from another a consecutive sequence of 0 to 4, preferably of 1 to 3, more preferably of 1 to 2 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof; and

wherein U/T represents uridine, or optionally thymidine;

stem2 [$N_{3-5}CN_{0-2}$]	is reverse complementary or partially reverse complementary with element stem1, and is a consecutive sequence between of 5 to 7 nucleotides;

wherein N_{3-5} is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

wherein N_{0-2} is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G or C or a nucleotide analogue thereof; and

wherein C is cytidine or an analogue thereof, and may be optionally replaced by a guanosine or an analogue thereof provided that its complementary nucleoside guanosine in stem1 is replaced by cytidine;

wherein stem1 and stem2 are capable of base pairing with each other forming a reverse complementary sequence, wherein base pairing may occur between stem1 and stem2, e.g. by Watson-Crick base pairing of nucleotides A and U/T or G and C or by non-Watson-Crick base pairing e.g. wobble base pairing, reverse Watson-Crick base pairing, Hoogsteen base pairing, reverse Hoogsteen base pairing or are capable of base pairing with each other forming a partially reverse complementary sequence, wherein an incomplete base pairing may occur between stem1 and stem2, on the basis that one or more bases in one stem do not have a complementary base in the reverse complementary sequence of the other stem.

According to a further preferred embodiment of the first inventive aspect, the inventive mRNA sequence may comprise at least one histone stem-loop sequence according to at least one of the following specific formulae (Ia) or (IIa):

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

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

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

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

wherein N, C, G, T and U are as defined above.

According to a further more particularly preferred embodiment of the first aspect, the at least one RNA may comprise at least one histone stem-loop sequence according to at least one of the following specific formulae (Ib) or (IIb):

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

$$\underbrace{[N_1GN_4]}_{\text{stem1}}\ \underbrace{[N_2(U/T)N_1]}_{\text{loop}}\ \underbrace{[N_4CN_1]}_{\text{stem2}}$$

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

$$\underbrace{N_{4\text{-}5}}_{\text{stem1 bordering element}}\ \underbrace{[N_1GN_4]}_{\text{stem1}}\ \underbrace{[N_2(U/T)N_1]}_{\text{loop}}\ \underbrace{[N_4CN_1]}_{\text{stem2}}\ \underbrace{N_{4\text{-}5}}_{\text{stem2 bordering element}}$$

wherein N, C, G, T and U are as defined above.

A particular preferred histone stem-loop sequence is the sequence according to SEQ ID No: 8.

More preferably the stem-loop sequence is the corresponding RNA sequence of the nucleic acid sequence according to SEQ ID NO: 9

Poly(A)

In a particularly preferred embodiment, the at least one RNA of the inventive composition comprises additionally to the coding region encoding at least one peptide or protein as described above or a fragment or variant thereof, a poly(A) sequence, also called poly-A tail, preferably at the 3' terminus of the RNA. When present, such a poly(A) sequence comprises a sequence of about 25 to about 400 adenosine nucleotides, preferably a sequence of about 50 to about 400 adenosine nucleotides, more preferably a sequence of about 50 to about 300 adenosine nucleotides, even more preferably a sequence of about 50 to about 250 adenosine nucleotides, most preferably a sequence of about 60 to about 250 adenosine nucleotides. In this context the term "about" refers to a deviation of ± 10% of the value(s) it is attached to. This poly(A) sequence is preferably located 3' of the coding region comprised in the RNA according to the invention.

Preferably, the poly(A) sequence in at least one RNA of the composition is derived from a DNA template by RNA in vitro transcription. Alternatively, the poly(A) sequence may also be obtained in vitro by common methods of chemical-synthesis without being necessarily transcribed from a DNA-progenitor. Moreover, poly(A) sequences, or poly(A) tails may be generated by enzymatic polyadenylation of the at least one RNA using commercially available polyadenylation kits and corresponding protocols known in the art.

Alternatively, the at least one RNA of the inventive composition optionally comprises a polyadenylation signal, which is defined herein as a signal, which conveys polyadenylation to a (transcribed) RNA by specific protein factors (e.g. cleavage and polyadenylation specificity factor (CPSF), cleavage stimulation factor (CstF), cleavage factors I and II (CF I and CF II), poly(A) polymerase (PAP)). In this context, a consensus polyadenylation signal is preferred comprising the NN(U/T)ANA consensus sequence. In a particularly preferred aspect, the polyadenylation signal comprises one of the following sequences: AA(U/T)AAA or A(U/T)(U/T)AAA (wherein uridine is usually present in RNA and thymidine is usually present in DNA).

Poly(C)

According to a further preferred embodiment, the RNA of the inventive composition can be modified by a sequence of at least 10 cytosines, preferably at least 20 cytosines, more preferably at

least 30 cytosines (so-called "poly(C) sequence"). Particularly, the RNA may contain a poly(C) sequence of typically about 10 to 200 cytosine nucleotides, preferably about 10 to 100 cytosine nucleotides, more preferably about 10 to 70 cytosine nucleotides or even more preferably about 20 to 50 or even 20 to 30 cytosine nucleotides. This poly(C) sequence is preferably located 3' of the coding region, more preferably 3' of an optional poly(A) sequence comprised in the RNA according to the present invention.

5'-Cap

According to another preferred embodiment of the invention, a modified RNA molecule as defined herein, can be modified by the addition of a so-called "5' cap" structure, which preferably stabilizes the RNA as described herein. A 5'-cap is an entity, typically a modified nucleotide entity, which generally "caps" the 5'-end of a mature mRNA. A 5'-cap may typically be formed by a modified nucleotide, particularly by a derivative of a guanine nucleotide. Preferably, the 5'-cap is linked to the 5'-terminus via a 5'-5'-triphosphate linkage. A 5'-cap may be methylated, e.g. m7GpppN, wherein N is the terminal 5' nucleotide of the nucleic acid carrying the 5'-cap, typically the 5'-end of an mRNA. m7GpppN is the 5'-cap structure, which naturally occurs in mRNA transcribed by polymerase II and is therefore preferably not considered as modification comprised in a modified RNA in this context. Accordingly, a modified RNA of the present invention may comprise a m7GpppN as 5'-cap, but additionally the modified RNA typically comprises at least one further modification as defined herein.

Further examples of 5'cap structures include glyceryl, inverted deoxy abasic residue (moiety), 4',5' methylene nucleotide, 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide, 1,5-anhydrohexitol nucleotide, L-nucleotides, alpha-nucleotide, modified base nucleotide, threo-pentofuranosyl nucleotide, acyclic 3',4'-seco nucleotide, acyclic 3,4-dihydroxybutyl nucleotide, acyclic 3,5 dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety, 3'-3'-inverted abasic moiety, 3'-2'-inverted nucleotide moiety, 3'-2'-inverted abasic moiety, 1,4-butanediol phosphate, 3'-phosphoramidate, hexylphosphate, aminohexyl phosphate, 3'-phosphate, 3'phosphorothioate, phosphorodithioate, or bridging or non-bridging methylphosphonate moiety. These modified 5'-cap structures are regarded as at least one modification in this context.

Particularly preferred modified 5'-cap structures are cap1 (methylation of the ribose of the adjacent nucleotide of m7G), cap2 (additional methylation of the ribose of the 2nd nucleotide downstream of the m7G), cap3 (additional methylation of the ribose of the 3rd nucleotide downstream of the m7G), cap4 (methylation of the ribose of the 4th nucleotide downstream of the m7G), ARCA (anti-reverse cap analogue, modified ARCA (e.g. phosphothioate modified ARCA), inosine, N1-methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

Secretory signal sequence:

According to another particularly preferred embodiment, the at least one RNA of the composition may additionally or alternatively encode a secretory signal peptide. Such secretory signal sequences are peptide stretches, which typically exhibit a length of about 15 to 30 amino acids and are preferably located at the N-terminus of the encoded peptide, without being limited thereto. Secretory signal sequences as defined herein preferably allow the transport of the encoded peptide or protein as encoded by the at least one coding sequence of the at least one RNA of the composition into a defined cellular compartiment, preferably the cell surface, the endoplasmic reticulum (ER) or the endosomal-lysosomal compartiment. Examples of secretory signal sequences as defined herein include, without being limited thereto, secretory signal sequences of classical or non-classical MHC-molecules (e.g. signal sequences of MHC I and II molecules, e.g. of the MHC class I molecule HLA-A*0201), secretory signal sequences of cytokines or immunoglobulines as defined herein, secretory signal sequences of the invariant chain of immunoglobulines or antibodies as defined herein, signal sequences of Lamp1, Tapasin, Erp57, Calretikulin, Calnexin, and further membrane associated proteins or of proteins associated with the endoplasmic reticulum (ER) or the endosomal-lysosomal compartiment.

Any of the above modifications regarding the coding sequence and/or regarding the RNA as defined above may be applied to the coding sequence and/or the RNA of the composition of the present invention, and further to any RNA as used in the context of 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 at least one RNA. A person skilled in the art will be able to take his choice accordingly.

Production of mRNA and RNA

The RNA may be prepared using any method known in the art, including synthetic methods (chemical synthesis of RNA) such as e.g. solid phase synthesis, as well as *in vitro* methods, such as RNA *in vitro* transcription reactions.

Combinations:

According to the present invention it is particularly preferred to combine RNA encoded peptides or proteins. In this context particularly preferred are the following combinations:

- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one chemokine
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at at least one suicide gene product
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one immunogenic protein or peptide
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one apoptosis inducer
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one angiogenesis inhibitor
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one heat shock protein
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one tumor antigen
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one β-catenin inhibitor
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one activator of the STING pathway
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one checkpoint modulator

- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one innate immune activator
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one antibody
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one decoy receptor
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one inhibitor of myeloid derived suppressor cells (MDSCs)
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one IDO pathway inhibitor
- RNA, preferably mRNA coding for at least one cytokine + RNA, preferably mRNA coding for at least one protein or peptide that bind inhibitors of apoptosis.

Furthermore, particularly preferred are the following embodiments:

- RNA, preferably mRNA coding for IL-2 and/or RNA, preferably mRNA coding for IL-12 + mRNA coding for thymidine kinase (approach: cytokines + suicide gene product)
- RNA, preferably mRNA coding for IL-2 and/or RNA, preferably mRNA coding for IL-12
- RNA, preferably mRNA coding for IL-12 and/or RNA, preferably mRNA coding for CD40L
- RNA, preferably mRNA coding for IL-15 and/or RNA, preferably mRNA coding for IL-12
- RNA, preferably mRNA coding for IL-2 + RNA, preferably mRNA coding for Influenza NP protein
- RNA, preferably mRNA coding for IL-2 and/or RNA, preferably mRNA coding for IL-12 + RNA, preferably mRNA coding for cytochrome c/caspase 3 (cytokines + apoptosis induction)
- RNA, preferably mRNA coding for CD40L + RNA, preferably mRNA coding for IL-12 + RNA, preferably mRNA coding for ΔRIGI

It has to be understood that the RNA molecules of the inventive composition may code for one or more different peptides or proteins (e.g. cytokines, chemokines, suicide gene products, immunogenic proteins or peptides, apoptosis inducers, angiogenesis inhibitors, heat shock proteins, tumor antigens, β-catenin inhibitors, activators of the STING pathway, checkpoint

modulators, innate immune activators, antibodies, dominant negative receptors and decoy receptors, inhibitors of myeloid derived suppressor cells (MDSCs), IDO pathway inhibitors, and proteins or peptides that bind inhibitors of apoptosis.as described above. Several RNA sequences may be combined in one inventive RNA containing composition. Moreover it is possible that the RNA sequence or sequences of the inventive composition code for variants or fragments of the wild type protein sequence or for one or more parts or fragments of the wild type protein sequence or variants thereof.

Non coding RNA

According to the invention the at least one RNA of the inventive RNA containing composition may comprise at least one non-coding RNA, which is preferably selected from the group consisting of small interfering RNA (siRNA), antisense RNA (asRNA), circular RNA (circRNA), ribozymes, aptamers, riboswitches, immunostimulating/immunostimulatory RNA RNA, transfer RNA (tRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), microRNA (miRNA), and Piwi-interacting RNA (piRNA).

Immunostimulatory/immunostimulating RNA (isRNA):

Likewise, according to a further alternative, the at least one RNA of the inventive RNA containing composition is an immunostimulatory/immunostimulating RNA, which preferably elicits an innate immune response. Such an immunostimulatory RNA may be any (double-stranded or single-stranded) RNA, e.g. a coding RNA, as defined herein. In a preferred embodiment, the immunostimulatory RNA is a non-coding RNA. Preferably, the immunostimulatory RNA may be a single-stranded, a double-stranded or a partially double-stranded RNA, more preferably a single-stranded RNA, and/or a circular or linear RNA, more preferably a linear RNA. More preferably, the immunostimulatory RNA may be a (linear) single-stranded RNA. Even more preferably, the immunostimulatory RNA may be a (long) (linear) single-stranded) non-coding RNA. In this context it is particular preferred that the isRNA carries a triphosphate at its 5'-end which is the case for in vitro transcribed RNA. An immunostimulatory RNA may also occur as a short RNA oligonucleotide as defined herein.

An immunostimulatory RNA as used herein may furthermore be selected from any class of RNA molecules, found in nature or being prepared synthetically, and which can induce an innate

immune response and may support an adaptive immune response induced by an antigen. In this context, an immune response may occur in various ways. A substantial factor for a suitable (adaptive) immune response is the stimulation of different T cell sub-populations. T-lymphocytes are typically divided into two sub-populations, the T-helper 1 (Th1) cells and the T-helper 2 (Th2) cells, with which the immune system is capable of destroying intracellular (Th1) and extracellular (Th2) pathogens (e.g. antigens). The two Th cell populations differ in the pattern of the effector proteins (cytokines) produced by them. Thus, Th1 cells assist the cellular immune response by activation of macrophages and cytotoxic T cells. Th2 cells, on the other hand, promote the humoral immune response by stimulation of B-cells for conversion into plasma cells and by formation of antibodies (e.g. against antigens). The Th1/Th2 ratio is therefore of great importance in the induction and maintenance of an adaptive immune response. In connection with the present invention, the Th1/Th2 ratio of the (adaptive) immune response is preferably shifted in the direction towards the cellular response (Th1 response) and a cellular immune response is thereby induced. According to one example, the innate immune system which may support an adaptive immune response may be activated by ligands of Toll-like receptors (TLRs). TLRs are a family of highly conserved pattern recognition receptor (PRR) polypeptides that recognize pathogen-associated molecular patterns (PAMPs) and play a critical role in innate immunity in mammals. Currently at least thirteen family members, designated TLR1 – TLR13 (Toll-like receptors: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12 or TLR13), have been identified. Furthermore, a number of specific TLR ligands have been identified. Furthermore, it has been reported that ligands for certain TLRs include certain nucleic acid molecules and that certain types of RNA are immunostimulatory in a sequence-independent or sequence-dependent manner, wherein these various immunostimulatory RNAs may e.g. stimulate TLR3, TLR7, or TLR8, or intracellular receptors such as RIG-I, MDA-5, etc.

Preferably, an immunostimulatory nucleic acid, preferably an immunostimulatory RNA (isRNA), as used herein, may comprise any RNA sequence known to be immunostimulatory, including, without being limited thereto, 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 selected from (human) family members TLR1 – TLR10, even 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. Furthermore, (classes of) immunostimulatory RNA molecules, used as a further compound of the inventive vaccine, may include any other RNA capable of eliciting an immune response. Without being limited thereto, such an immunostimulatory RNA may include ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNA (mRNA), and viral RNA (vRNA). 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.

An immunostimulatory RNA as used herein may furthermore be selected from any class of RNA molecules, found in nature or being prepared synthetically, and which can induce an innate immune response and may support an adaptive immune response induced by an antigen. In this context, an immune response may occur in various ways. A substantial factor for a suitable (adaptive) immune response is the stimulation of different T-cell sub-populations. T-lymphocytes are typically divided into two sub-populations, the T-helper 1 (Th1) cells and the T-helper 2 (Th2) cells, with which the immune system is capable of destroying intracellular (Th1) and extracellular (Th2) pathogens (e.g. antigens). The two Th cell populations differ in the pattern of the effector proteins (cytokines) produced by them. Thus, Th1 cells assist the cellular immune response by activation of macrophages and cytotoxic T-cells. Th2 cells, on the other hand, promote the humoral immune response by stimulation of B-cells for conversion into plasma cells and by formation of antibodies (e.g. against antigens). The Th1/Th2 ratio is therefore of great importance in the induction and maintenance of an adaptive immune response. In connection with the present invention, the Th1/Th2 ratio of the (adaptive) immune response is preferably shifted in the direction towards the cellular response (Th1 response) and a cellular immune response is thereby induced. According to one example, the innate immune system which may support an adaptive immune response, may be activated by ligands of Toll-like receptors (TLRs). TLRs are a family of highly conserved pattern recognition receptor (PRR) polypeptides that recognize pathogen-associated molecular patterns (PAMPs) and play a critical role in innate immunity in mammals. Currently at least thirteen family members, designated TLR1 – TLR13 (Toll-like receptors: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12 or TLR13), have been identified. Furthermore, a number of specific TLR ligands have been identified. It was e.g. found that unmethylated bacterial DNA and synthetic analogs thereof (CpG DNA) are ligands for TLR9 (Hemmi H et al. (2000) Nature 408:740-5; Bauer S et al. (2001) Proc NatlAcadSci USA 98, 9237-42).

Furthermore, it has been reported that ligands for certain TLRs include certain nucleic acid molecules and that certain types of RNA are immunostimulatory in a sequence-independent or sequence-dependent manner, wherein these various immunostimulatory RNAs may e.g. stimulate TLR3, TLR7, or TLR8, or intracellular receptors such as RIG-I, MDA-5, etc. E.g. Lipford et al. determined certain G,U-containing oligoribonucleotides as immunostimulatory by acting via TLR7 and TLR8 (see WO 03/086280). The immunostimulatory G,U-containing oligoribonucleotides described by Lipford et al. were believed to be derivable from RNA sources including ribosomal RNA, transfer RNA, messenger RNA, and viral RNA.

According to a particularly preferred embodiment, such immunostimulatory nucleic acid sequences is preferably RNA preferably consisting of or comprising a nucleic acid of the following formula (III) or (IV):

GlXmGn , (formula (III))

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.

ClXmCn , (formula (IV))

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 (II) or (III), which may be used as immunostimulatory RNA may be relatively short nucleic acid molecules with a typical 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 inventive nucleic acid cargo complex 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 (III) 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 Gl or Gn can be, for example, a GUGU, GGUU, UGUG, UUGG, GUUG, GGGU, GGUG, GUGG, UGGG or GGGG, etc.; when l or n = 5 Gl or Gn 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 Xm in the nucleic acid of formula (III) according to the invention is preferably not a uracil. Similarly, the number of nucleotides C in the

nucleic acid of formula (IV) 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, Cl or Cn can be, for example, a CUCU, CCUU, UCUC, UUCC, CUUC, CCCU, CCUC, CUCC, UCCC or CCCC, etc.; when l or n = 5 Cl or Cn 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 Xm in the nucleic acid of formula (III) according to the invention is preferably not a uracil. Preferably, for formula (II), 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 G1 and/or Gn 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 (III).

According to a particularly preferred embodiment, a nucleic acid according to any of formulas (III) or (IV) above, which may be used as immunostimulatory RNA, may be selected from a sequence consisting or comprising any of the following sequences SEQ ID NOs 298 – 381.

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

According to a further particularly preferred embodiment, such immunostimulatory nucleic acid sequences particularly isRNA consist of or comprise a nucleic acid of formula (V) or (VI):

(NuGlXmGnNv)a , (formula (V))

wherein:

G is guanosine (guanine), uridine (uracil) or an analogue of guanosine (guanine) or uridine (uracil), preferably guanosine (guanine) or an analogue thereof;

X is guanosine (guanine), uridine (uracil), adenosine (adenine), thymidine (thymine), cytidine (cytosine), or an analogue of these nucleotides (nucleosides), preferably uridine (uracil) or an analogue thereof;

N is a nucleic acid sequence having a length of about 4 to 50, preferably of about 4 to 40, more preferably of about 4 to 30 or 4 to 20 nucleic acids, each N independently being selected from guanosine (guanine), uridine (uracil), adenosine (adenine), thymidine (thymine), cytidine (cytosine) or an analogue of these nucleotides (nucleosides);

a is an integer from 1 to 20, preferably from 1 to 15, most preferably from 1 to 10;

l is an integer from 1 to 40,

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

when l > 1, at least 50% of these nucleotides (nucleosides) are guanosine (guanine) or an analogue thereof;

m is an integer and is at least 3;

wherein when m = 3, X is uridine (uracil) or an analogue thereof, and

when m > 3, at least 3 successive uridines (uracils) or analogues of uridine (uracil) occur;

n is an integer from 1 to 40,

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

when n > 1, at least 50% of these nucleotides (nucleosides) are guanosine (guanine) or an analogue thereof;

u,v may be independently from each other an integer from 0 to 50,

preferably wherein when u = 0, v ≥ 1, or

when v = 0, u ≥ 1;

wherein the nucleic acid molecule of formula (IV) has a length of at least 50 nucleotides, preferably of at least 100 nucleotides, more preferably of at least 150 nucleotides, even more preferably of at least 200 nucleotides and most preferably of at least 250 nucleotides.

(NuClXmCnNv)a , (formula (VI))

wherein:

C is cytidine (cytosine), uridine (uracil) or an analogue of cytidine (cytosine) or uridine (uracil), preferably cytidine (cytosine) or an analogue thereof;

X is guanosine (guanine), uridine (uracil), adenosine (adenine), thymidine (thymine), cytidine (cytosine) or an analogue of the above-mentioned nucleotides (nucleosides), preferably uridine (uracil) or an analogue thereof;

N is each a nucleic acid sequence having independent from each other a length of about 4 to 50, preferably of about 4 to 40, more preferably of about 4 to 30 or 4 to 20 nucleic acids, each N independently being selected from guanosine (guanine), uridine (uracil), adenosine (adenine), thymidine (thymine), cytidine (cytosine) or an analogue of these nucleotides (nucleosides);

a is an integer from 1 to 20, preferably from 1 to 15, most preferably from 1 to 10;

l is an integer from 1 to 40,

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

when l > 1, at least 50% of these nucleotides (nucleosides) are cytidine (cytosine) or an analogue thereof;

m is an integer and is at least 3;

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

when m > 3, at least 3 successive uridines (uracils) or analogues of uridine (uracil) occur;

n is an integer from 1 to 40,

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

when n > 1, at least 50% of these nucleotides (nucleosides) are cytidine (cytosine) or an analogue thereof.

u, v may be independently from each other an integer from 0 to 50,

preferably wherein when u = 0, $v \geq 1$, or

when v = 0, $u \geq 1$;

wherein the nucleic acid molecule of formula (V) according to the invention has a length of at least 50 nucleotides, preferably of at least 100 nucleotides, more preferably of at least 150 nucleotides, even more preferably of at least 200 nucleotides and most preferably of at least 250 nucleotides.

For formula (VI), any of the definitions given above for elements N (i.e. Nu and Nv) and X (Xm), particularly the core structure as defined above, as well as for integers a, l, m, n, u and v, similarly apply to elements of formula (VI) correspondingly, wherein in formula (VI) the core structure is

defined by ClXmCn. The definition of bordering elements Nu and Nv is identical to the definitions given above for Nu and Nv.

According to a very particularly preferred embodiment, the nucleic acid molecule, preferably immunostimulating RNA according to formula (V) may be selected from e.g. any of the sequences according to SEQ ID NOs 382-395 or from a sequence having at least 60%, 70%, 80%, 90%, or even 95% sequence identity with any of these sequences.

In this context particularly preferred are immunostimulating RNAs according to SEQ ID NOs 5, 394 and 10072.

R2025:

GGGAGAAAGCUCAAGCUUAUCCAAGUAGGCUGGUCACCUGUACAACGUAGCCGGUAUUUU
UUUUUUUUUUUUUUUUUUGACCGUCUCAAGGUCCAAGUUAGUCUGCCUAUAAAGGUGCGG
AUCCACAGCUGAUGAAAGACUUGUGCGGUACGGUUAAUCUCCCCUUUUUUUUUUUUUUUU
UUUUUAGUAAAUGCGUCUACUGAAUCCAGCGAUGAUGCUGGCCCAGAUCUUCGACCACAA
GUGCAUAUAGUAGUCAUCGAGGGUCGCCUUUUUUUUUUUUUUUUUUUUUUGGCCCAGUU
CUGAGACUUCGCUAGAGACUACAGUUACAGCUGCAGUAGUAACCACUGCGGCUAUUGCAG
GAAAUCCCGUUCAGGUUUUUUUUUUUUUUUUUUUUUCCGCUCACUAUGAUUAAGAACCAG
GUGGAGUGUCACUGCUCUCGAGGUCUCACGAGAGCGCUCGAUACAGUCCUUGGAAGAAUC
UUUUUUUUUUUUUUUUUUUUUUUGUGCGACGAUCACAGAGAACUUCUAUUCAUGCAGGUCU
GCUCUAG (SEQ ID NO: 5 and 394)

R3630:

GGGAGAAAGCUCAAGCUUAUCCAAGUAGGCUGGUCACCUGUACAACGUAGCCGGUAUUUU
UUUUUUUUUUUUUUUUUUGACCGUCUCAAGGUCCAAGUUAGUCUGCCUAUAAAGGUGCGG
AUCCACAGCUGAUGAAAGACUUGUGCGGUACGGUUAAUCUCCCCUUUUUUUUUUUUUUUU
UUUUUAGUAAAUGCGUCUACUGAAUCCAGCGAUGAUGCUGGCCCAGAUCUUCGACCACAA
GUGCAUAUAGUAGUCAUCGAGGGUCGCCUUUUUUUUUUUUUUUUUUUUUUGGCCCAGUU
CUGAGACUUCGCUAGAGACUACAGUUACAGCUGCAGUAGUAACCACUGCGGCUAUUGCAG
GAAAUCCCGUUCAGGUUUUUUUUUUUUUUUUUUUUUCCGCUCACUAUGAUUAAGAACCAG
GUGGAGUGUCACUGCUCUCGAGGUCUCACGAGAGCGCUCGAUACAGUGCUUGGAAGAAUC
UUUUUUUUUUUUUUUUUUUUUUUGUGCGACGAUCACAGAGAACUUCUAUUCAUGCAGGUCU

GCUCUAG (SEQ ID NO. 10072).

According to another very particularly preferred embodiment, the nucleic acid molecule according to formula (VI) may be selected from e.g. any of the sequences according to SEQ ID NO 396 or 397, or from a sequence having at least 60%, 70%, 80%, 90%, or even 95% sequence identity with any of these sequences.

All modifications disclosed in the context of coding RNA may also be applied in the context of non-coding RNA if applicable.

Combination of coding and non-coding RNA

In particularly preferred embodiments the inventive RNA containing composition comprises at least one RNA encoding at least one peptide or protein and at least one non-coding RNA as defined above, preferably at least one immunostimulating RNA.

Particularly preferred are the following embodiments:

- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one cytokine, preferably IL-2, IL-12, IL-15 or CD40L
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one chemokine
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one suicide gene product, preferably Herpes simplex virus thymidine kinase
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one immunogenic protein or peptide, preferably Influenza NP protein
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one apoptosis inducer, preferably cytochrome c or caspase 3
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one angiogenesis inducer

- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one heat shock protein
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one tumor antigen
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one β-catenin inhibitor
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one activator of the STING pathway
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one checkpoint modulator, preferably an antibody directed against PD-1, PD-L1 or CTLA4
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one innate immune activator, preferably a constitutive active variant of RIG-1
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one antibody
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one decoy receptor, preferably a soluble PD-1 receptor
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one inhibitor of myeloid derived suppressor cells (MDSCs)
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one IDO pathway inhibitor
- Immostimulating RNA preferably according to SEQ ID Nos XY or YY + RNA, preferably mRNA coding for at least one protein or peptide that bind inhibitors of apoptosis.

More particularly preferred are the following embodiments:

- Immostimulating RNA preferably according SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one cytokine, preferably IL-2, IL-12, IL-15 or CD40L + RNA, preferably mRNA coding for at least one immunogenic protein or peptide, preferably Influenza NP protein

- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one cytokine, preferably IL-2, IL-12, IL-15 or CD40L + RNA, preferably mRNA coding for at least one innate immune activator, preferably a constitutive active variant of RIG-1
- Immostimulating RNA preferably according to SEQ ID Nos 5, 394, or 10072 + RNA, preferably mRNA coding for at least one cytokine, preferably IL-2, IL-12, or IL-15, + RNA, preferably mRNA coding for at least one further cytokine, preferably CD40L.

Formulation and Complexation

The at least one RNA of the inventive composition may be administered naked without being associated with any further vehicle, carrier, transfection or complexation agent.

In a preferred embodiment, the RNA of the inventive composition is formulated together with further compounds for increasing the transfection efficiency and/or the immunostimulatory properties of the RNA. Such compounds are termed herein carriers, vehicles, transfection or complexation agents. Preferably, the RNA is formulated together with one or more cationic or polycationic compounds, preferably with cationic or polycationic polymers, cationic or polycationic peptides or proteins, cationic or polycationic polysaccharides, cationic or polycationic lipids and/or with a polymeric carrier. Such cationic or polycationic polymers, cationic or polycationic peptides or proteins, cationic or polycationic polysaccharides, cationic or polycationic lipids or polymeric carriers are useful as carriers, vehicles, transfection or complexation agents of nucleic acids in the context of the present invention. Accordingly, in a further embodiment of the invention it is preferred that the at least one RNA or any other nucleic acid comprised in the inventive composition is associated with or complexed with a cationic or polycationic compound or a polymeric carrier, optionally in a weight ratio selected from a range of about 6:1 (w/w) to about 0.25:1 (w/w), more preferably from about 5:1 (w/w) to about 0.5:1 (w/w), even more preferably of about 4:1 (w/w) to about 1:1 (w/w) or of about 3:1 (w/w) to about 1:1 (w/w), and most preferably a ratio of about 3:1 (w/w) to about 2:1 (w/w) of RNA or nucleic acid to cationic or polycationic compound and/or with a polymeric carrier; or optionally in a nitrogen/phosphate ratio of RNA or nucleic acid to cationic or polycationic compound and/or polymeric carrier in the range of about

0.1-10, preferably in a range of about 0.3-4 or 0.3-1, and most preferably in a range of about 0.5-1 or 0.7-1, and even most preferably in a range of about 0.3-0.9 or 0.5-0.9.

The ratio of the at least one RNA as described above, and the cationic or polycationic compound, may be calculated on the basis of the nitrogen/phosphate ratio (N/P-ratio) of all these components. In the context of the present invention, an N/P-ratio is preferably in the range of about 0.01-4, 0.01-2, 0.1-2 or 0.1-1.5 regarding the ratio of nucleic acids: cationic or polycationic peptide contained in the inventive vaccine, and most preferably in the range of about 0.1-1. Such an N/P ratio is preferably designed to provide good transfection properties *in vivo* and transport into and through cell membranes. Preferably, for this purpose, cationic or polycationic compound and/or polymeric carriers as used herein, are based on peptide sequences.

Cationic or polycationic compounds, being particularly preferred agents in this context include 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, 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, proline-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.

In this context protamine is particularly preferred.

Additionally, preferred cationic or polycationic proteins or peptides may be selected from the following proteins or peptides having the following total formula (VII):

$$(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x, \qquad \text{(formula (VII)}$$

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 cationic peptides in this context are e.g. Arg_7, Arg_8, Arg_9, H_3R_9, R_9H_3, $H_3R_9H_3$, $YSSR_9SSY$, $(RKH)_4$, $Y(RKH)_2R$, etc. In this context the disclosure of WO 2009/030481 is incorporated herewith by reference.

A polymeric carrier used according to the invention might be a polymeric carrier formed by disulfide-crosslinked cationic components.

According to a further particularly preferred embodiment, cationic or polycationic peptides or proteins of the polymeric carrier, having the empirical sum formula (VII) as shown above and which comprise or are additionally modified to comprise at least one –SH moeity, may be, without being restricted thereto, selected from the subgroup consisting of generic formulas Arg7 (also termed as R7), Arg9 (also termed R9), Arg12 (also termed as R12).

According to a one further particularly preferred embodiment, the cationic or polycationic peptide or protein of the polymeric carrier, when defined according to formula {(Arg)l;(Lys)m;(His)n;(Orn)o;(Xaa)x} (formula (VII)) as shown above and which comprise or are additionally modified to comprise at least one –SH moeity, may be, without being restricted thereto, selected from subformula (VIIa):

{(Arg)l;(Lys)m;(His)n;(Orn)o;(Xaa')x (Cys)y} formula (VIIa)

wherein (Arg)l;(Lys)m;(His)n;(Orn)o; and x are as defined herein, Xaa' is any amino acid selected from native (= naturally occurring) or non-native amino acids except of Arg, Lys, His, Orn or Cys and y is any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80 and 81-90, provided that the overall content of Arg (Arginine), Lys (Lysine), His (Histidine) and Orn (Ornithine) represents at least 10% of all amino acids of the oligopeptide.

This embodiment may apply to situations, wherein the cationic or polycationic peptide or protein of the polymeric carrier, e.g. when defined according to empirical formula (Arg)l;(Lys)m;(His)n;(Orn)o;(Xaa)x (formula (VII)) as shown above, comprises or has been modified with at least one cysteine as –SH moiety in the above meaning such that the cationic or

polycationic peptide as cationic component carries at least one cysteine, which is capable to form a disulfide bond with other components of the polymeric carrier.

Exemplary examples may comprise any of the following sequences:

Cys(Arg7), Cys(Arg8), Cys(Arg9), Cys(Arg10), Cys(Arg11), Cys(Arg12), Cys(Arg13), Cys(Arg14), Cys(Arg15), Cys(Arg16), Cys(Arg17), Cys(Arg18), Cys(Arg19), Cys(Arg20).

According to another particularly preferred embodiment, the cationic or polycationic peptide or protein of the polymeric carrier, when defined according to formula {(Arg)l;(Lys)m;(His)n;(Orn)o;(Xaa)x} (formula (VII)) as shown above, may be, without being restricted thereto, selected from subformula (VIIb):

Cys1 {(Arg)l;(Lys)m;(His)n;(Orn)o;(Xaa)x} Cys2 (formula (VIIb))

wherein empirical formula {(Arg)l;(Lys)m;(His)n;(Orn)o;(Xaa)x} (formula (VII)) is as defined herein and forms a core of an amino acid sequence according to (semiempirical) formula (I) and wherein Cys1 and Cys2 are Cysteines proximal to, or terminal to (Arg)l;(Lys)m;(His)n;(Orn)o;(Xaa)x.

Exemplary examples may comprise any of the above sequences flanked by two Cys and following sequences:

Cys(Arg7)Cys, Cys(Arg8)Cys, Cys(Arg9)Cys, Cys(Arg10)Cys, Cys(Arg11)Cys, Cys(Arg12)Cys, Cys(Arg13)Cys, Cys(Arg14)Cys, Cys(Arg15)Cys, Cys(Arg16)Cys, Cys(Arg17)Cys, Cys(Arg18)Cys, Cys(Arg19)Cys, Cys(Arg20)Cys (SEQ ID NOs: 10-23):

This embodiment may apply to situations, wherein the cationic or polycationic peptide or protein of the polymeric carrier, e.g. when defined according to empirical formula (Arg)l;(Lys)m;(His)n;(Orn)o;(Xaa)x (formula (VII)) as shown above, has been modified with at least two cysteines as –SH moieties in the above meaning such that the cationic or polycationic peptide of the inventive polymeric carrier cargo complex as cationic component carries at least two (terminal) cysteines, which are capable to form a disulfide bond with other components of the polymeric carrier.

In a preferred embodiment, the polymeric carrier is formed by, comprises or consists of the peptide CysArg12Cys (SEQ ID NO: 15) or CysArg12 (CRRRRRRRRRRR).

According to a second alternative, at least one cationic (or polycationic) component of the polymeric carrier may be selected from e.g. any (non-peptidic) cationic or polycationic polymer suitable in this context, provided that this (non-peptidic) cationic or polycationic polymer exhibits or is modified to exhibit at least one –SH-moiety, which provide for a disulfide bond linking the cationic or polycationic polymer with another component of the polymeric carrier as defined herein. Thus, likewise as defined herein, the polymeric carrier may comprise the same or different cationic or polycationic polymers.

In the specific case that the cationic component of the polymeric carrier comprises a (non-peptidic) cationic or polycationic polymer the cationic properties of the (non-peptidic) cationic or polycationic polymer may be determined upon its content of cationic charges when compared to the overall charges of the components of the cationic polymer. Preferably, the content of cationic charges in the cationic polymer at a (physiological) pH as defined herein is at least 10%, 20%, or 30%, preferably at least 40%, more preferably at least 50%, 60% or 70%, but also preferably at least 80%, 90%, or even 95%, 96%, 97%, 98%, 99% or 100%, most preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, or may be in the range of about 10% to 90%, more preferably in the range of about 30% to 100%, even preferably in the range of about 50% to 100%, e.g. 50, 60, 70, 80%, 90% or 100%, or in a range formed by any two of the afore mentioned values, provided, that the content of all charges, e.g. positive and negative charges at a (physiological) pH as defined herein, in the entire cationic polymer is 100%.

Preferably, the (non-peptidic) cationic component of the polymeric carrier represents a cationic or polycationic polymer, typically exhibiting a molecular weight of about 0.1 or 0.5 kDa to about 100 kDa, preferably of about 1 kDa to about 75 kDa, more preferably of about 5 kDa to about 50 kDa, even more preferably of about 5 kDa to about 30 kDa, or a molecular weight of about 10 kDa to about 50 kDa, even more preferably of about 10 kDa to about 30 kDa. Additionally, the (non-peptidic) cationic or polycationic polymer typically exhibits at least one -SH-moiety, which is capable to form a disulfide linkage upon condensation with either other cationic components or other components of the polymeric carrier as defined herein.

In the above context, the (non-peptidic) cationic component of the polymeric carrier may be selected from acrylates, modified acrylates, such as pDMAEMA (poly(dimethylaminoethyl methylacrylate)), chitosanes, aziridines or 2-ethyl-2-oxazoline (forming oligo ethylenimines or modifed oligoethylenimines), polymers obtained by reaction of bisacrylates with amines forming oligo beta aminoesters or poly amido amines, or other polymers like polyesters, polycarbonates, etc. Each molecule of these (non-peptidic) cationic or polycationic polymers typically exhibits at least one –SH-moiety, wherein these at least one –SH-moiety may be introduced into the (non-peptidic) cationic or polycationic polymer by chemical modifications, e.g. using imonothiolan, 3-thio propionic acid or introduction of –SH-moieties containing amino acids, such as cysteine or any further (modified) amino acid. Such –SH-moieties are preferably as already defined above.

The disulfide-crosslinked cationic components may be the same or different from each other. The polymeric carrier can also contain further components. It is also particularly preferred that the polymeric carrier used according to the present invention comprises mixtures of cationic peptides, proteins or polymers and optionally further components as defined herein, which are crosslinked by disulfide bonds as described herein. In this context the disclosure of WO 2012/013326 is incorporated herewith by reference.

In this context the cationic components, which form basis for the polymeric carrier by disulfide-crosslinkage, are typically selected from any suitable cationic or polycationic peptide, protein or polymer suitable for this purpose, particular any cationic or polycationic peptide, protein or polymer capable to complex an RNA or a nucleic acid as defined according to the present invention, and thereby preferably condensing the RNA or the nucleic acid. The cationic or polycationic peptide, protein or polymer, is preferably a linear molecule, however, branched cationic or polycationic peptides, proteins or polymers may also be used.

Every disulfide-crosslinking cationic or polycationic protein, peptide or polymer of the polymeric carrier, which may be used to complex the RNA of the inventive composition or any further nucleic acid comprised in the inventive composition contains at least one –SH moiety, most preferably at least one cysteine residue or any further chemical group exhibiting an –SH moiety, capable to form a disulfide linkage upon condensation with at least one further cationic or polycationic protein, peptide or polymer as cationic component of the polymeric carrier as mentioned herein.

As defined above, the polymeric carrier, which may be used to complex the RNA of the inventive composition or any further nucleic acid comprised in the inventive composition may be formed by disulfide-crosslinked cationic (or polycationic) components.

Nucleic acids complexed with such polymeric carriers are also termed herein as "polymeric carrier cargo complexes".

In this context it is particularly preferred that if immunostimulating RNA is used in the context of the present invention that this immunostimulating RNA is complexed with a polymeric carrier as defined above. Preferably, the immunostimulating RNA, (e.g. comprising an RNA sequence according to any of formulae III-VI), most preferably comprising an RNA sequence according to SEQ ID NOs. 5, 394, or 10072, is complexed with a polymeric carrier comprising or formed by disulfide-crosslinked peptides according to formula VII, VIIa or VIIb, preferably a polymeric carrier formed by Cys(Arg12)Cys or Cys(Arg12). Such a particularly preferred embodiment is termed herein also as "RNAdjuvant".

In a further particular embodiment, the polymeric carrier which may be used to complex the RNA or any further nucleic acid comprised in the inventive composition may be selected from a polymeric carrier molecule according to generic formula (VIII):

$$L\text{-}P^1\text{-}S\text{-}[S\text{-}P^2\text{-}S]_n\text{-}S\text{-}P^3\text{-}L \qquad \text{formula (VIII)}$$

wherein,

P^1 and P^3 are different or identical to each other and represent a linear or branched hydrophilic polymer chain, each P^1 and P^3 exhibiting at least one –SH-moiety, capable to form a disulfide linkage upon condensation with component P^2, or alternatively with (AA), $(AA)_x$, or $[(AA)_x]_z$ if such components are used as a linker between P^1 and P^2 or P^3 and P^2 and/or with further components (e.g. (AA), $(AA)_x$, $[(AA)_x]_z$ or L), the linear or branched hydrophilic polymer chain selected independent from each other from polyethylene glycol (PEG), poly-*N*-(2-hydroxypropyl)methacrylamide, poly-2-(methacryloyloxy)ethyl phosphorylcholines, poly(hydroxyalkyl L-asparagine), poly(2-(methacryloyloxy)ethyl phosphorylcholine), hydroxyethylstarch or poly(hydroxyalkyl L-glutamine), wherein the hydrophilic polymer chain exhibits a molecular weight of about 1 kDa to about 100 kDa,

preferably of about 2 kDa to about 25 kDa; or more preferably of about 2 kDa to about 10 kDa, e.g. about 5 kDa to about 25 kDa or 5 kDa to about 10 kDa;

P^2 is a cationic or polycationic peptide or protein, e.g. as defined above for the polymeric carrier formed by disulfide-crosslinked cationic components, and preferably having a length of about 3 to about 100 amino acids, more preferably having a length of about 3 to about 50 amino acids, even more preferably having a length of about 3 to about 25 amino acids, e.g. a length of about 3 to 10, 5 to 15, 10 to 20 or 15 to 25 amino acids, more preferably a length of about 5 to about 20 and even more preferably a length of about 10 to about 20; or

is a cationic or polycationic polymer, e.g. as defined above for the polymeric carrier formed by disulfide-crosslinked cationic components, typically having a molecular weight of about 0.5 kDa to about 30 kDa, including a molecular weight of about 1 kDa to about 20 kDa, even more preferably of about 1.5 kDa to about 10 kDa, or having a molecular weight of about 0.5 kDa to about 100 kDa, including a molecular weight of about 10 kDa to about 50 kDa, even more preferably of about 10 kDa to about 30 kDa;

each P^2 exhibiting at least two –SH-moieties, capable to form a disulfide linkage upon condensation with further components P^2 or component(s) P^1 and/or P^3 or alternatively with further components (e.g. (AA), $(AA)_x$, or $[(AA)_x]_z$);

-S-S- is a (reversible) disulfide bond (the brackets are omitted for better readability), wherein S preferably represents sulphur or a –SH carrying moiety, which has formed a (reversible) disulfide bond. The (reversible) disulfide bond is preferably formed by condensation of –SH-moieties of either components P^1 and P^2, P^2 and P^2, or P^2 and P^3, or optionally of further components as defined herein (e.g. L, (AA), $(AA)_x$, $[(AA)_x]_z$, etc); The –SH-moiety may be part of the structure of these components or added by a modification as defined below;

L is an optional ligand, which may be present or not, and may be selected independent from the other from RGD, Transferrin, Folate, a signal peptide or signal sequence, a localization signal or sequence, a nuclear localization signal or sequence (NLS), an antibody, a cell penetrating peptide, (e.g. TAT or KALA), a ligand of a receptor (e.g.

cytokines, hormones, growth factors etc), small molecules (e.g. carbohydrates like mannose or galactose or synthetic ligands), small molecule agonists, inhibitors or antagonists of receptors (e.g. RGD peptidomimetic analogues), or any further protein as defined herein, etc.;

n is an integer, typically selected from a range of about 1 to 50, preferably from a range of about 1, 2 or 3 to 30, more preferably from a range of about 1, 2, 3, 4, or 5 to 25, or a range of about 1, 2, 3, 4, or 5 to 20, or a range of about 1, 2, 3, 4, or 5 to 15, or a range of about 1, 2, 3, 4, or 5 to 10, including e.g. a range of about 4 to 9, 4 to 10, 3 to 20, 4 to 20, 5 to 20, or 10 to 20, or a range of about 3 to 15, 4 to 15, 5 to 15, or 10 to 15, or a range of about 6 to 11 or 7 to 10. Most preferably, n is in a range of about 1, 2, 3, 4, or 5 to 10, more preferably in a range of about 1, 2, 3, or 4 to 9, in a range of about 1, 2, 3, or 4 to 8, or in a range of about 1, 2, or 3 to 7.

In this context the disclosure of WO 2011/026641 and WO 2012/116811 is incorporated herewith by reference. Each of hydrophilic polymers P^1 and P^3 typically exhibits at least one –SH-moiety, wherein the at least one –SH-moiety is capable to form a disulfide linkage upon reaction with component P^2 or with component (AA) or $(AA)_x$, if used as linker between P^1 and P^2 or P^3 and P^2 as defined below and optionally with a further component, e.g. L and/or (AA) or $(AA)_x$, e.g. if two or more –SH-moieties are contained. The following subformulae "P^1-S-S-P^2" and "P^2-S-S-P^3" within the generic formula above, wherein any of S, P^1 and P^3 are as defined herein, typically represent a situation, wherein one –SH-moiety of hydrophilic polymers P^1 and P^3 was condensed with one –SH-moiety of component P^2 of the generic formula above, wherein both sulphurs of these –SH-moieties form a disulfide bond –S-S-. These –SH-moieties are typically provided by each of the hydrophilic polymers P^1 and P^3, e.g. via an internal cysteine or any further (modified) amino acid or compound which carries a –SH moiety. Accordingly, the subformulae "P^1-S-S-P^2" and "P^2-S-S-P^3" may also be written as "P^1-Cys-Cys-P^2" and "P^2-Cys-Cys-P^3", if the –SH- moiety is provided by a cysteine, wherein the term Cys-Cys represents two cysteines coupled via a disulfide bond, not via a peptide bond. In this case, the term "-S-S-" in these formulae may also be written as "-S-Cys", as "-Cys-S" or as "-Cys-Cys-". In this context, the term "-Cys-Cys-" does not represent a peptide bond but a linkage of two cysteines via their –SH-moieties to form a disulfide bond. Accordingly, the term "-Cys-Cys-" also may be understood generally as "-(Cys-S)-(S-Cys)-", wherein in this specific case S

indicates the sulphur of the –SH-moiety of cysteine. Likewise, the terms "-S-Cys" and "-Cys-S" indicate a disulfide bond between a –SH containing moiety and a cysteine, which may also be written as "-S-(S-Cys)" and "-(Cys-S)-S". Alternatively, the hydrophilic polymers P^1 and P^3 may be modified with a –SH moiety, preferably via a chemical reaction with a compound carrying a –SH moiety, such that each of the hydrophilic polymers P^1 and P^3 carries at least one such –SH moiety. Such a compound carrying a –SH moiety may be e.g. an (additional) cysteine or any further (modified) amino acid, which carries a –SH moiety. Such a compound may also be any non-amino compound or moiety, which contains or allows to introduce a –SH moiety into hydrophilic polymers P^1 and P^3 as defined herein. Such non-amino compounds may be attached to the hydrophilic polymers P^1 and P^3 of the polymeric carrier via chemical reactions or binding of compounds, e.g. by binding of a 3-thio propionic acid or thioimolane, by amide formation (e.g. carboxylic acids, sulphonic acids, amines, etc), by Michael addition (e.g maleinimide moieties, unsatured carbonyls, etc), by click chemistry (e.g. azides or alkines), by alkene/alkine methatesis (e.g. alkenes or alkines), imine or hydrozone formation (aldehydes or ketons, hydrazins, hydroxylamins, amines), complexation reactions (avidin, biotin, protein G) or components which allow S_n-type substitution reactions (e.g halogenalkans, thiols, alcohols, amines, hydrazines, hydrazides, sulphonic acid esters, oxyphosphonium salts) or other chemical moieties which can be utilized in the attachment of further components. A particularly preferred PEG derivate in this context is alpha-Methoxy-omega-mercapto poly(ethylene glycol). In each case, the SH-moiety, e.g. of a cysteine or of any further (modified) amino acid or compound, may be present at the terminal ends or internally at any position of hydrophilic polymers P^1 and P^3. As defined herein, each of hydrophilic polymers P^1 and P^3 typically exhibits at least one –SH-moiety preferably at one terminal end, but may also contain two or even more –SH-moieties, which may be used to additionally attach further components as defined herein, preferably further functional peptides or proteins e.g. a ligand, an amino acid component (AA) or $(AA)_x$, antibodies, cell penetrating peptides or enhancer peptides (e.g. TAT, KALA), etc.

As defined above, ligands (L), may be optionally used in the polymeric carrier molecule according to generic formula (VIII), e.g. for direction of the inventive carrier polymer and its entire "cargo" (the adjuvant component and/or the antigen of the inventive composition or vaccine composition) into specific cells. They may be selected independent from the other from RGD, Transferrin, Folate, a signal peptide or signal sequence, a localization signal or sequence, a nuclear localization signal or

sequence (NLS), an antibody, a cell penetrating peptide (CPP), (e.g. TAT, KALA), a ligand of a receptor (e.g. cytokines, hormones, growth factors etc), small molecules (e.g. carbohydrates like mannose or galactose or synthetic ligands), small molecule agonists, inhibitors or antagonists of receptors (e.g. RGD peptidomimetic analogues) or any such molecule as further defined below, etc. Particularly preferred are cell penetrating peptides (CPPs), which induce a pH-mediated conformational change in the endosome and lead to an improved release of the inventive polymeric carrier (in complex with a nucleic acid) from the endosome by insertion into the lipid layer of the liposome. Such called CPPs or cationic peptides for transportation, may include, without being limited thereto protamine, nucleoline, spermine or spermidine, poly-L-lysine (PLL), basic polypeptides, poly-arginine, chimeric CPPs, such as Transportan, or MPG peptides, HIV-binding peptides, Tat, HIV-1 Tat (HIV), Tat-derived peptides, oligoarginines, members of the penetratin family, e.g. Penetratin, Antennapedia-derived peptides (particularly from *Drosophila antennapedia*), pAntp, pIsl, etc., antimicrobial-derived CPPs e.g. Buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, MAP, PpTG20, Proline-rich peptides, Loligomers, Arginine-rich peptides, Calcitonin-peptides, FGF, Lactoferrin, , poly-L-Lysine, poly-Arginine, histones, VP22 derived or analog peptides, Pestivirus Erns, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs, PpT620, prolin-rich peptides, arginine-rich peptides, lysine-rich peptides, Pep-1, L-oligomers, Calcitonin peptide(s), etc. Particularly preferred in this context is mannose as ligand to target antigen presenting cells which carries on their cell membrane mannose receptors. In a further preferred aspect of the first embodiment of the present invention galactose as optional ligand can be used to target hepatocytes. Such ligands may be attached to component P^1 and/or P^3 by reversible disulfide bonds as defined below or by any other possible chemical attachement, e.g. by amide formation (e.g. carboxylic acids, sulphonic acids, amines, etc), by Michael addition (e.g. maleinimide moieties, α, β unsatured carbonyls, etc), by click chemistry (e.g. azides or alkines), by alkene/alkine methatesis (e.g. alkenes or alkines), imine or hydrozone formation (aldehydes or ketons, hydrazins, hydroxylamins, amines), complexation reactions (avidin, biotin, protein G) or components which allow S_n-type substitution reactions (e.g halogenalkans, thiols, alcohols, amines, hydrazines, hydrazides, sulphonic acid esters, oxyphosphonium salts) or other chemical moieties which can be utilized in the attachment of further components.

In the context of formula (VIII) of the present invention components P^1 and P^3 represent a linear or branched hydrophilic polymer chain, containing at least one –SH-moiety, each P^1 and P^3

independently selected from each other, e.g. from polyethylene glycol (PEG), poly-*N*-(2-hydroxypropyl)methacrylamide, poly-2-(methacryloyloxy)ethyl phosphorylcholines, poly(hydroxyalkyl L-asparagine) or poly(hydroxyalkyl L-glutamine). P^1 and P^3 may be identical or different to each other. Preferably, each of hydrophilic polymers P^1 and P^3 exhibits a molecular weight of about 1 kDa to about 100 kDa, preferably of about 1 kDa to about 75 kDa, more preferably of about 5 kDa to about 50 kDa, even more preferably of about 5 kDa to about 25 kDa. Additionally, each of hydrophilic polymers P^1 and P^3 typically exhibits at least one –SH-moiety, wherein the at least one –SH-moiety is capable to form a disulfide linkage upon reaction with component P^2 or with component (AA) or $(AA)_x$, if used as linker between P^1 and P^2 or P^3 and P^2 as defined below and optionally with a further component, e.g. L and/or (AA) or $(AA)_x$, e.g. if two or more –SH-moieties are contained. The following subformulae "P^1-S-S-P^2" and "P^2-S-S-P^3" within generic formula (VII) above (the brackets are omitted for better readability), wherein any of S, P^1 and P^3 are as defined herein, typically represent a situation, wherein one–SH-moiety of hydrophilic polymers P^1 and P^3 was condensed with one –SH-moiety of component P^2 of generic formula (VII) above, wherein both sulphurs of these –SH-moieties form a disulfide bond –S-S- as defined herein in formula (VII). These –SH-moieties are typically provided by each of the hydrophilic polymers P^1 and P^3, e.g. via an internal cysteine or any further (modified) amino acid or compound which carries a –SH moiety. Accordingly, the subformulae "P^1-S-S-P^2" and "P^2-S-S-P^3" may also be written as "P^1-Cys-Cys-P^2" and "P^2-Cys-Cys-P^3", if the –SH- moiety is provided by a cysteine, wherein the term Cys-Cys represents two cysteines coupled via a disulfide bond, not via a peptide bond. In this case, the term "-S-S-" in these formulae may also be written as "-S-Cys", as "-Cys-S" or as "-Cys-Cys-". In this context, the term "-Cys-Cys-" does not represent a peptide bond but a linkage of two cysteines via their –SH-moieties to form a disulfide bond. Accordingly, the term "-Cys-Cys-" also may be understood generally as "-(Cys-S)-(S-Cys)-", wherein in this specific case S indicates the sulphur of the –SH-moiety of cysteine. Likewise, the terms "-S-Cys" and "-Cys-S" indicate a disulfide bond between a –SH containing moiety and a cysteine, which may also be written as "-S-(S-Cys)" and "-(Cys-S)-S". Alternatively, the hydrophilic polymers P^1 and P^3 may be modified with a –SH moiety, preferably via a chemical reaction with a compound carrying a –SH moiety, such that each of the hydrophilic polymers P^1 and P^3 carries at least one such –SH moiety. Such a compound carrying a –SH moiety may be e.g. an (additional) cysteine or any further (modified) amino acid, which carries a –SH moiety. Such a compound may also be any non-amino compound or moiety, which contains or allows to introduce a –SH moiety into hydrophilic polymers P^1 and P^3 as defined herein. Such non-

amino compounds may be attached to the hydrophilic polymers P^1 and P^3 of formula (VII) of the polymeric carrier according to the present invention via chemical reactions or binding of compounds, e.g. by binding of a 3-thio propionic acid or thioimolane, by amide formation (e.g. carboxylic acids, sulphonic acids, amines, etc), by Michael addition (e.g maleinimide moieties, α, β unsatured carbonyls, etc), by click chemistry (e.g. azides or alkines), by alkene/alkine methatesis (e.g. alkenes or alkines), imine or hydrozone formation (aldehydes or ketons, hydrazins, hydroxylamins, amines), complexation reactions (avidin, biotin, protein G) or components which allow S_n-type substitution reactions (e.g halogenalkans, thiols, alcohols, amines, hydrazines, hydrazides, sulphonic acid esters, oxyphosphonium salts) or other chemical moieties which can be utilized in the attachment of further components. A particularly preferred PEG derivate in this context is alpha-Methoxy-omega-mercapto poly(ethylene glycol). In each case, the SH-moiety, e.g. of a cysteine or of any further (modified) amino acid or compound, may be present at the terminal ends or internally at any position of hydrophilic polymers P^1 and P^3. As defined herein, each of hydrophilic polymers P^1 and P^3 typically exhibits at least one –SH-moiety preferably at one terminal end, but may also contain two or even more –SH-moieties, which may be used to additionally attach further components as defined herein, preferably further functional peptides or proteins e.g. a ligand, an amino acid component (AA) or $(AA)_x$, antibodies, cell penetrating peptides or enhancer peptides (e.g. TAT, KALA), etc.

According to one preferred alternative, such further functional peptides or proteins may comprise so called cell penetrating peptides (CPPs) or cationic peptides for transportation. Particularly preferred are CPPs, which induce a pH-mediated conformational change in the endosome and lead to an improved release of the inventive polymeric carrier (in complex with a nucleic acid) from the endosome by insertion into the lipid layer of the liposome. Such called cell penetrating peptides (CPPs) or cationic peptides for transportation, may include, without being limited thereto protamine, nucleoline, spermine or spermidine, poly-L-lysine (PLL), basic polypeptides, poly-arginine, chimeric CPPs, such as Transportan, or MPG peptides, HIV-binding peptides, Tat, HIV-1 Tat (HIV), Tat-derived peptides, oligoarginines, members of the penetratin family, e.g. Penetratin, Antennapedia-derived peptides (particularly from *Drosophila antennapedia*), pAntp, plsl, etc., antimicrobial-derived CPPs e.g. Buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, MAP, PpTG20, Proline-rich peptides, Loligomers, Arginine-rich peptides, Calcitonin-peptides, FGF, Lactoferrin, , poly-L-Lysine, poly-Arginine, histones, VP22 derived or analog peptides,

Pestivirus Erns, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs, PpT620, prolin-rich peptides, arginine-rich peptides, lysine-rich peptides, Pep-1, L-oligomers, Calcitonin peptide(s), etc.

According to a further preferred embodiment of the present invention, each of hydrophilic polymers P^1 and P^3 of formula (VIII) of the polymeric carrier used according to the present invention may also contain at least one further functional moiety, which allows attaching further components as defined herein, e.g. a ligand as defined above, or functionalities which allow the attachment of further components, e.g. by amide formation (e.g. carboxylic acids, sulphonic acids, amines, etc), by Michael addition (e.g maleinimide moieties, unsatured carbonyls, etc), by click chemistry (e.g. azides or alkines), by alkene/alkine methatesis (e.g. alkenes or alkines), imine or hydrozone formation (aldehydes or ketons, hydrazins, hydroxylamins, amines), complexation reactions (avidin, biotin, protein G) or components which allow S_n-type substitution reactions (e.g halogenalkans, thiols, alcohols, amines, hydrazines, hydrazides, sulphonic acid esters, oxyphosphonium salts) or other chemical moieties which can be utilized in the attachment of further components. Further functional moieties may comprise an amino acid component (AA) as defined herein or $(AA)_x$., wherein (AA) is preferably an amino component as defined above. In the above context, x is preferably an integer and may be selected from a range of about 1 to 100, preferably from a range of about 1 to 50, more preferably 1 to 30, and even more preferably selected from a number comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15-30, e.g. from a range of about 1 to 30, from a range of about 1 to 15, or from a number comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, or may be selected from a range formed by any two of the afore mentioned values. Most preferably, x is 1. Such an amino acid component (AA) or $(AA)_x$ may be contained in every part of the inventive polymeric carrier according to formula (VIII) above and therefore may be attached to all components of the inventive polymeric carrier according to formula (VII). It is particularly preferred that amino acid component (AA) or $(AA)_x$ is present as a ligand or part of the repetitive component $[S\text{-}P^2\text{-}S]_n$ within formula (VIII) of the inventive polymeric carrier.

In the context of the entire formula (VIII) of the polymeric carrier may be preferably defined as follows:

$L\text{-}P^1\text{-}S\text{-}[Cys\text{-}P^2\text{-}Cys]_n\text{-}S\text{-}P^3\text{-}L$

wherein L, P^1, P^2, P^3 and n are as defined herein, S is sulphur and each Cys provides for one –SH-moiety for the disulfide bond.

According to a particular embodiment, the polymeric carrier according to formula (VII) as defined above, may comprise at least one amino acid component (AA) or $(AA)_x$, as defined above. Such an amino acid component (AA) or $(AA)_x$ may be contained in every part of the inventive polymeric carrier according to formula (VIII) above and therefore may be attached to all components of the polymeric carrier according to formula (VIII). It is particularly preferred that amino acid component (AA) or $(AA)_x$ is present as a ligand or part of the repetitive component $[S\text{-}P^2\text{-}S]_n$ within formula (VIII) of the polymeric carrier. The amino acid component (AA) or $(AA)_x$ preferably contains or is flanked (e.g. terminally) by at least one –SH containing moiety, which allows introducing this component (AA) or $(AA)_x$ via a disulfide bond into the polymeric carrier according to formula (VIII) as defined herein. Such a –SH-containing moiety may be any –SH containing moiety (or, of course, one sulphur of a disulfide bond), e.g. a cysteine residue. In the specific case that the –SH containing moiety represents a cysteine, the amino acid component $(AA)_x$ may also be read as -Cys-$(AA)_x$- or -Cys-$(AA)_x$-Cys- wherein Cys represents Cysteine and provides for the necessary –SH-moiety for a disulfide bond. The –SH containing moiety may be also introduced into the amino acid component $(AA)_x$ using any of modifications or reactions as shown above for components P^1, P^2 or P^3. In the specific case that the amino acid component $(AA)_x$ is linked to two components of the polymeric carrier according to formula (VIII) it is preferred that (AA) or $(AA)_x$ contains at least two –SH-moieties, e.g. at least two Cysteines, preferably at its terminal ends. This is particularly preferred if (AA) or $(AA)_x$ is part of the repetitive component $[S\text{-}P^2\text{-}S]_n$. Alternatively, the amino acid component (AA) or $(AA)_x$ is introduced into the polymeric carrier according to formula (VIII) as defined herein via any chemical possible addition reaction. Therefore the amino acid component (AA) or $(AA)_x$ contains at least one further functional moiety, which allows attaching same to a further component as defined herein, e.g. component P^1 or P^3, P^2, L, or a further amino acid component (AA) or $(AA)_x$, etc. Such functional moieties may be selected from functionalities which allow the attachment of further components, e.g. functionalities as defined herein, e.g. by amide formation (e.g. carboxylic acids, sulphonic acids, amines, etc), by Michael addition (e.g maleinimide moieties, α, β unsatured carbonyls, etc), by click chemistry (e.g. azides or alkines), by alkene/alkine

methatesis (e.g. alkenes or alkines), imine or hydrozone formation (aldehydes or ketons, hydrazins, hydroxylamins, amines), complexation reactions (avidin, biotin, protein G) or components which allow S_n-type substitution reactions (e.g halogenalkans, thiols, alcohols, amines, hydrazines, hydrazides, sulphonic acid esters, oxyphosphonium salts) or other chemical moieties which can be utilized in the attachment of further components.

The amino acid component (AA) or $(AA)_x$ in the polymeric carrier of formula (VIII) may also occur as a mixed repetitive amino acid component $[(AA)_x]_z$, wherein the number of amino acid components (AA) or $(AA)_x$ is further defined by integer z. In this context, z may be selected from a range of about 1 to 30, preferably from a range of about 1 to 15, more preferably 1 to 10 or 1 to 5 and even more preferably selected from a number selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, or may be selected from a range formed by any two of the afore mentioned values.

According to a specific and particularly preferred alternative, the amino acid component (AA) or $(AA)_x$, preferably written as S-$(AA)_x$-S or [S-$(AA)_x$-S] may be used to modify component P^2, particularly the content of component S-P^2-S in repetitive component [S-P^2-S]$_n$ of the polymeric carrier of formula (VIII) above. This may be represented in the context of the entire polymeric carrier according to formula (VIII) e.g. by following formula (VIIIa):

$$L\text{-}P^1\text{-}S\text{-}\{[S\text{-}P^2\text{-}S]_a[S\text{-}(AA)_x\text{-}S]_b\}\text{-}S\text{-}P^3\text{-}L, \quad \text{(VIIIa)}$$

wherein x, S, L, AA, P^1, P^2 and P^3 are preferably as defined herein. In formula (VIIIa) above, any of the single components [S-P^2-S] and [S-$(AA)_x$-S] may occur in any order in the subformula {[S-P^2-S]$_a$[S-$(AA)_x$-S]$_b$}. The numbers of single components [S-P^2-S] and [S-$(AA)_x$-S] in the subformula {[S-P^2-S]$_a$[S-$(AA)_x$-S]$_b$} are determined by integers a and b, wherein a + b = n. n is an integer and is defined as above for formula (VIII).

a is an integer, typically selected independent from integer b from a range of about 1 to 50, preferably from a range of about 1, 2 or 3 to 30, more preferably from a range of about 1, 2, 3, 4, or 5 to 25, or a range of about 1, 2, 3, 4, or 5 to 20, or a range of about 1, 2, 3, 4, or 5 to 15, or a range of about 1, 2, 3, 4, or 5 to 10, including e.g. a range of about 3 to 20, 4 to 20, 5 to 20, or 10 to 20, or a range of about 3 to 15, 4 to 15, 5 to 15, or 10 to 15, or a range of about 6 to 11 or 7 to 10. Most

preferably, a is in a range of about 1, 2, 3, 4, or 5 to 10, more preferably in a range of about 1, 2, 3, or 4 to 9, in a range of about 1, 2, 3, or 4 to 8, or in a range of about 1, 2, or 3 to 7.

b is an integer, typically selected independent from integer a from a range of about 0 to 50 or 1 to 50, preferably from a range of about 0, 1, 2 or 3 to 30, more preferably from a range of about 0, 1, 2, 3, 4, or 5 to 25, or a range of about 0, 1, 2, 3, 4, or 5 to 20, or a range of about 0, 1, 2, 3, 4, or 5 to 15, or a range of about 0, 1, 2, 3, 4, or 5 to 10, including e.g. a range of about 3 to 20, 4 to 20, 5 to 20, or 10 to 20, or a range of about 3 to 15, 4 to 15, 5 to 15, or 10 to 15, or a range of about 6 to 11 or 7 to 10. Most preferably, b is in a range of about 1, 2, 3, 4, or 5 to 10, more preferably in a range of about 1, 2, 3, or 4 to 9, in a range of about 1, 2, 3, or 4 to 8, or in a range of about 1, 2, or 3 to 7.

In this context it is particularly preferred that the RNA, preferably mRNA of the inventive composition is complexed at least partially with a cationic or polycationic compound and/or a polymeric carrier, preferably cationic proteins or peptides. In this context the disclosure of WO 2010/037539 and WO 2012/113513 is incorporated herewith by reference. Partially means that only a part of the RNA is complexed with a cationic compound and that the rest of the RNA is (comprised in the inventive composition) in uncomplexed form ("free"). Preferably the ratio of complexed RNA to: free RNA (in the inventive composition) is selected from a range of about 5:1 (w/w) to about 1:10 (w/w), more preferably from a range of about 4:1 (w/w) to about 1:8 (w/w), even more preferably from a range of about 3:1 (w/w) to about 1:5 (w/w) or 1:3 (w/w), and most preferably the ratio of complexed RNA to free RNA in the inventive composition is selected from a ratio of about 1:1 (w/w).

The so called "(adjuvant) component", which may be used to together with the RNA, preferably mRNA in the inventive composition, is preferably prepared according to a first step by complexing the at least one (m)RNA of the (adjuvant) component with a cationic or polycationic compound and/or with a polymeric carrier, preferably as defined herein, in a specific ratio to form a stable complex. In this context, it is highly preferable, that no free cationic or polycationic compound or polymeric carrier or only a neglectably small amount thereof remains in the (adjuvant) component after complexing the (m)RNA. Accordingly, the ratio of the (m)RNA and the cationic or polycationic compound and/or the polymeric carrier in the (adjuvant) component is typically selected in a range

that the (m)RNA is entirely complexed and no free cationic or polycationic compound or polymeric carrier or only a neglectably small amount thereof remains in the composition. Preferably the ratio of the (adjuvant) component, i.e. the ratio of the (m)RNA to the cationic or polycationic compound and/or the polymeric carrier, preferably as defined herein, is selected from a range of about 6:1 (w/w) to about 0,25:1 (w/w), more preferably from about 5:1 (w/w) to about 0,5:1 (w/w), even more preferably of about 4:1 (w/w) to about 1:1 (w/w) or of about 3:1 (w/w) to about 1:1 (w/w), and most preferably a ratio of about 3:1 (w/w) to about 2:1 (w/w). Alternatively, the ratio of the (m)RNA to the cationic or polycationic compound and/or the polymeric carrier, preferably as defined herein, in the (adjuvant) component, may also be calculated on the basis of the nitrogen/phosphate ratio (N/P-ratio) of the entire complex. In the context of the present invention, an N/P-ratio is preferably in the range of about 0.1-10, preferably in a range of about 0.3-4 and most preferably in a range of about 0.5-2 or 0.7-2 regarding the ratio of RNA: cationic or polycationic compound and/or polymeric carrier, preferably as defined herein, in the complex, and most preferably in the range of about 0.7-1.5, preferably provided the cationic or polycationic compound in the complex is a cationic or polycationic cationic or polycationic protein or peptide and/or the polymeric carrier is as defined herein. Such ratios, particularly weight and/or N/P ratios may also be applied to ratios of the at least one RNA as defined herein to a cationic or polycationic polymer or a polymeric carrier as defined herein used to complex the at least one RNA.

In this context, the N/P ratio is a measure of the ionic charge of the cationic (side chain) component of the cationic or polycationic compound or. In particular, if the cationic properties of the cationic compound are generated by nitrogens (e.g. of the amino acid side chains), the N/P ratio expresses the ratio of basic nitrogen atoms to phosphate residues in the nucleotide backbone, considering that (side chain) nitrogen atoms in the cationic compound contribute to positive charges and phosphate of the phosphate backbone of the nucleic acid contribute to the negative charge. The N/P-ratio is defined as the nitrogen/phosphate ratio (N/P-ratio) of the entire complex of nucleic acid and cationic or polycationic compound. This is typically illustrative for the content/amount of cationic compounds and characteristic for the content/amount of nucleic acids bound or complexed. It may be calculated on the basis that, for example, 1 µg RNA typically contains about 3 nmol phosphate residues, provided that RNA exhibits a statistical distribution of bases. Additionally, 1 nmol peptide typically contains about x nmol nitrogen residues, dependent on the molecular weight and the number of its (cationic) amino acids.

According to a particularly preferred embodiment the inventive composition comprises a polymeric carrier cargo complex comprising or consisting of

a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components preferably as defined above, more preferably according to formula VII, VIIa, VIIb or VIII, and

b) as a cargo at least one nucleic acid molecule, preferably an immunostimulating RNA, most preferably an RNA comprising an RNA sequence according to SEQ ID NOs. 5, 394, or 10072,

preferably for use as a medicament, more preferably for use as an immunostimulating agent or adjuvant, preferably for the treatment of cancer or tumor diseases, wherein the polymeric carrier cargo complex is preferably administered intratumorally.

In a preferred embodiment, the inventive RNA containing composition comprises a polymeric carrier cargo complex, comprising:

a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, preferably as defined above, more preferably according to formula VII, VIIa, VIIb or VIII, and

a) b) as a cargo at least one first nucleic acid molecule, preferably an immunostimulating RNA, most preferably an RNA comprising an RNA sequence according to SEQ ID NOs. 5, 394, or 10072,

for use as an immunostimulating agent or as an adjuvant,

and at least one second nucleic acid molecule, preferably an RNA and more preferably an mRNA encoding at least one protein or a peptide most preferably as disclosed above for coding RNA, and wherein the inventive composition is preferably administered intratumorally.

In a preferred embodiment, the invention relates to a polymeric carrier cargo complex, comprising:

a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, preferably as defined above, more preferably according to formula VII, VIIa, VIIb or VIII, and

a) b) as a cargo at least one first nucleic acid molecule, preferably an immunostimulating RNA, most preferably an RNA comprising an RNA sequence according to SEQ ID NOs. 5, 394, or 10072,

for use as an immunostimulating agent or as an adjuvant,

wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule, preferably an RNA and more preferably an mRNA encoding at least one protein or a peptide most preferably as disclosed above for coding RNA, and

wherein the polymeric carrier cargo complex and the second nucleic acid molecule are preferably administered intratumorally.

Such preferred combinations of at least one first nucleic acid, preferably an immunostimulating RNA and at least one second nucleic acid, preferably an RNA, and more preferably an mRNA encoding at least one protein or peptide are disclosed above in the context of "combinations of coding and non-coding RNA".

As used herein, the term "first nucleic acid molecule" refers to a nucleic molecule, which is used as a cargo in the polymeric carrier cargo complex and is thus associated with the polymeric carrier. The term "second nucleic acid molecule", as used herein, typically refers to a nucleic acid, which is not part of the polymeric carrier cargo complex and which encodes at least one peptide or protein.

In the context of the present invention immunostimulating agents or adjuvants are understood as compounds, which are preferably efficient in inducing an innate immune response, particularly in inducing the anti-viral cytokine IFN-alpha.

Adjuvants or immunostimulating agents usually act via their capability to induce an innate immune response. The innate immune system forms the dominant system of host defense in most organisms and comprises barriers such as humoral and chemical barriers including, e.g., inflammation, the complement system and cellular barriers. The innate immune system is typically based on a small number of receptors, called pattern recognition receptors. They recognize conserved molecular patterns that distinguish foreign organisms, like viruses, bacteria, fungi and parasites, from cells of the host. Such pathogen-associated molecular patterns (PAMP) include viral nucleic acids, components of bacterial and fungal walls, flagellar proteins, and more. The first

family of pattern recognition receptors (PAMP receptors) studied in detail was the Toll-like receptor (TLR) family. TLRs are transmembrane proteins which recognize ligands of the extracellular milieu or of the lumen of endosomes. Following ligand-binding they transduce the signal via cytoplasmic adaptor proteins which leads to triggering of a host-defence response and entailing production of antimicrobial peptides, proinflammatory chemokines and cytokines, antiviral cytokines, etc. (see e.g. Meylan, E., J. Tschopp, et al. (2006), Nature 442(7098): 39-44). Further relevant components of the immune system include e.g. the endosomal TLRs, cytoplasmic receptors, Type I interferons and cytoplasmic receptors. Therefore, the immunostimulating agents or adjuvants are defined herein preferably as inducers of an innate immune response, which activate pattern recognition receptors (PAMP receptors). Hereby, a cascade of signals is elicited, which e.g. may result in the release of cytokines (e.g. IFN-alpha) supporting the innate immune response. Accordingly, it is preferably a feature of an immunostimulating agent or adjuvant to bind to such receptors and activate such PAMP receptors. Ideally, such as an agent or adjuvant additionally supports the adaptive immune response by e.g. shifting the immune response such that the preferred class of Th cells is activated. Depending on the disease or disorder to be treated a shift to a Th1-based immune response may be preferred or, in other cases, a shift to a Th2 immune response may be preferred. Furthermore, adjuvants are usually defined as compounds that can increase and/or modulate the intrinsic immunogenicity of an antigen.

The term "immunostimulating agent" is typically understood not to include agents as e.g. antigens (of whatever chemical structure), which elicit an adaptive/cytotoxic immune response, e.g. a "humoral" or "cellular" immune response, in other words elicit immune reponses (and confer immunity by themselves) which are characterized by a specific response to structural properties of an antigen recognized to be foreign by immune competent cells. Rather "immunostimulating agent"is typically understood to mean agents/compounds/complexes which do not trigger any adaptive immune response by themselves, but which may exlusively enhance such an adaptive immune reponse in an unspecific way, by e.g. activating "PAMP" receptors and thereby triggering the release of cytokines which support the actual adaptive immune response. Accordingly, any immunostimulation by agents (e.g. antigens) which evoke an adaptive immune response by themselves (conferring immunity by themselves directly or indirectly) is typically disclaimed by the phrase "immunostimulating agent".

The term "adjuvant" is also understood not to comprise agents which confer immunity by themselves. Accordingly, adjuvants do not by themselves confer immunity, but assist the immune system in various ways to enhance the antigen-specific immune response by e.g. promoting presentation of an antigen to the immune system. Hereby, an adjuvant may preferably e.g. modulate the antigen-specific immune response by e.g. shifting the dominating Th2-based antigen specific response to a more Th1-based antigen specific response or vice versa. Accordingly, the terms "immunostimulating agent" and "adjuvant" in the context of the present invention are typically understood to mean agents, compounds or complexes which do not confer immunity by themselves, but exclusively support the immune reponse in an unspecific way (in contrast to an antigen-specific immune response) by effects, which modulate the antigen-specific (adaptive cellular and/or humoral immune response) by unspecific measures, e.g. cytokine expression/secretion, improved antigen presentation, shifting the nature of the arms of the immune response etc.. Accordingly, any agents evoking by themselves immunity are typically disclaimed by the terms "adjuvant" or "immunostimulating agent".

The use of the polymeric carrier cargo complex optionally in combination with a second nucleic acid molecule, preferably an RNA, allows provision of a more efficient and/or safer medicament. Advantageously, the polymeric carrier cargo complex is suited for *in vivo* delivery of nucleic acids, in particular for compacting and stabilizing a nucleic acid for the purposes of nucleic acid transfection, such as exhibiting one or more reduced negative side effects of high-molecular weight polymers as discussed above, such as poor biodegradability or high toxicity, agglomeration, low transfection activity *in vivo*, etc. The polymeric carrier cargo complex also provides for improved nucleic acid transfer *in vivo*, particularly via intratumoral routes, including serum stability, salt stability, efficiency of uptake, reduced complement activation, nucleic acid release, etc. Such a polymeric carrier cargo complex furthermore may support induction and maintenance of an adaptive immune response by initiating or boosting a parallel innate immune response. It has been found that an improved adaptive immune response can further be obtained, in particular when the polymeric carrier cargo complex is administered in combination with a second nucleic acid molecule, preferably an RNA, encoding a protein or peptide, or when the polymeric carrier cargo complex is co-formulated in a pharmaceutical composition with a second nucleic acid molecule, preferably an RNA, encoding a protein or peptide, preferably an antigenic peptide or protein. It has proven as particularly beneficial in this respect to administer the inventive composition comprising the

polymeric carrier cargo complex optionally in combination with the second nucleic acid molecule as defined herein via an intratumoral route. Additionally, the polymeric carrier cargo complex may exhibit improved storage stability, particularly during lyophilisation.

In particular embodiments, the polymeric carrier cargo complex as defined above enhances the immune response against a protein or peptide, which is encoded by a second nucleic acid molecule, preferably an RNA, more preferably an mRNA, that is administered in combination with the polymeric carrier cargo complex, preferably via an intratumoral route of administration.

The polymeric carrier cargo complex and/or the second nucleic acid molecule encoding a peptide or protein are preferably provided together with 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 material, which is mixed with the polymeric carrier cargo complex and/or the second nucleic acid molecule. If the polymeric carrier cargo complex and/or the second nucleic acid molecule are 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. Ringer or Ringer-Lactate solution is particularly preferred as a liquid basis.

The phrase "administered in combination" as used herein refers to a situation, where the polymeric carrier cargo complex is administered to a subject before, concomittantly or after the administration of the second nucleic acid molecule encoding a protein or peptide to the same subject. Preferably, the time interval between the administration of the polymeric carrier cargo complex and the at least one second nucleic acid molecule, preferably an RNA, encoding a protein or peptide is less than about 48 hours, more preferably less than about 24 hours, 12 hours, 6 hours, 4 hours, 2 hours, 1 hour, most preferably less than about 30 minutes, 15 minutes or 5 minutes. In a particularly preferred embodiment, the phrase "administered in combination" refers to concomitant administration of the polymeric carrier cargo complex and the at least one second nucleic acid molecule, i.e. the simultaneous administration of both components or the administration of both components within a time frame that typically comprises less than 5 minutes. The phrase "administered in combination" does not only refer to a situation, where the pharmaceutical carrier cargo complex is in physical contact with the at least one second nucleic acid molecule or formulated together with said second nucleic acid molecule. The phrase

"administered in combination" as used herein comprises also the separate administration of the polymeric carrier cargo complex and the second nucleic acid molecule (e.g. by two separate injections), as long as the time interval between the two administrations does not exceed the interval as defined above. Alternatively, the polymeric carrier cargo complex and the second nucleic acid molecule may be administered in combination by mixing the polymeric carrier cargo complex and the second nucleic acid molecule prior to administration and administering the mixture to a subject. When the polymeric carrier cargo complex is formulated together with the second nucleic acid molecule or when a composition as defined herein is used, the polymeric carrier cargo complex and the second nucleic acid molecule may further, independently from each other, administered in combination via any of the administration routes as described herein.

The polymeric carrier cargo complex comprises as a cargo at least one nucleic acid molecule. In the context of the present invention, such a nucleic acid molecule may be any suitable nucleic acid, selected e.g. from any (single-stranded or double-stranded) DNA, preferably, without being limited thereto, e.g. genomic DNA, single-stranded DNA molecules, double-stranded DNA molecules, coding DNA, DNA primers, DNA probes, immunostimulatory/immunostimulating DNA, a (short) DNA oligonucleotide ((short) oligodesoxyribonucleotides), viral DNA, or may be selected e.g. from any PNA (peptide nucleic acid) or may be selected e.g. from any (single-stranded or double-stranded) RNA, preferably, without being limited thereto, a (short) RNA oligonucleotide ((short) oligoribonucleotide), a coding RNA, a messenger RNA (mRNA), a viral RNA, replicons, an immunostimulatory/immunostimulating RNA, a small interfering RNA (siRNA), an antisense RNA, a micro RNA, a small nuclear RNA (snRNA), a small-hairpin (sh) RNA or riboswitches, ribozymes or aptamers; etc. The nucleic acid molecule of the polymeric carrier cargo complex may also be a ribosomal RNA (rRNA), a transfer RNA (tRNA), a messenger RNA (mRNA), or a viral RNA (vRNA). Preferably, the nucleic acid molecule of the polymeric carrier cargo complex is an RNA. More preferably, the nucleic acid molecule of the polymeric carrier cargo complex is a (linear) single-stranded RNA, even more preferably an mRNA or an immunostimulatory/immunostimulating RNA.

Furthermore, the nucleic acid of the polymeric carrier cargo complex may be a single- or a double-stranded nucleic acid molecule 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 acid molecules are typically formed by a longer and a shorter

single-stranded nucleic acid molecule or by two single stranded nucleic acid molecules, which are about equal in length, wherein one single-stranded nucleic acid molecule is in part complementary to the other single-stranded nucleic acid molecule and both thus form a double-stranded nucleic acid molecule in this region, i.e. a partially double-stranded or partially single stranded nucleic acid molecule. Preferably, the nucleic acid molecule may be a single-stranded nucleic acid molecule. Furthermore, the nucleic acid molecule may be a circular or linear nucleic acid molecule, preferably a linear nucleic acid molecule.

According to one alternative, the nucleic acid molecule of the inventive polymeric carrier cargo complex may be a coding nucleic acid, e.g. a DNA or RNA. Moreover, the polymeric carrier cargo complex may be administered in combination with at least one second nucleic acid molecule, which encodes a protein or a peptide.

According to one embodiment, the at least one first nucleic acid molecule and the at least one second nucleic acid molecule are both coding nucleic acid molecules. Preferably, the at least one first and the at least one second nucleic acid molecule each encode a different peptide or protein. In one embodiment, the first nucleic acid molecule has a sequence, which is distinct from the sequence of the second nucleic acid molecule, which is administered in combination with the polymeric carrier cargo complex. Alternatively, the first nucleic acid molecule and the second nucleic acid molecule may comprise the same sequence or be identical.

In the case of the at least one first nucleic acid molecule and/or of the second nucleic acid molecule, such a coding DNA or RNA may be any DNA or RNA as defined herein. Preferably, such a coding DNA or RNA may be a single- or a double-stranded DNA or RNA, more preferably a single-stranded DNA or RNA, and/or a circular or linear DNA or RNA, more preferably a linear DNA or RNA. Furthermore such a coding DNA or RNA may be a genomic DNA, a viral RNA or DNA, a replicon, a plasmid DNA or an mRNA. Even more preferably, the coding DNA or RNA may be a (linear) single-stranded DNA or RNA. Most preferably, the nucleic acid molecule according to the present invention may be a linear single-stranded messenger RNA (mRNA). Such 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.

In a preferred embodiment, the at least one second nucleic acid molecule encodes a therapeutically active protein or an antigen as defined herein, preferably as disclosed in the context of "coding RNA". In a particularly preferred embodiment, the at least one second nucleic acid molecule, which is administered in combination with the polymeric carrier cargo complex, encodes a peptide or a protein, which is capable of eliciting an immune response, preferably an adaptive immune response, after administration, especially intratumoral administration, to a host. Alternatively, the at least one second nucleic acid molecule encodes at least one therapeutically active peptide or protein, preferably selected from the group consisting of cytokines, chemokines, suicide gene products, immunogenic proteins or peptides, apoptosis inducers, angiogenesis inhibitors, heat shock proteins, tumor antigens, β-catenin inhibitors, activators of the STING pathway, checkpoint modulators, innate immune activators, antibodies, dominant negative receptors and decoy receptors, inhibitors of myeloid derived suppressor cells (MDSCs), IDO pathway inhibitors, and proteins or peptides that bind inhibitors of apoptosis.

In a particular embodiment, the first nucleic acid molecule of the herein defined polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may contain backbone modifications, sugar modifications or base modifications. 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 of the inventive polymeric carrier cargo complex are chemically modified. A sugar modification in connection with the present invention is a chemical modification of the sugar of the nucleotides of the first nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. Furthermore, a base modification in connection with the present invention is a chemical modification of the base moiety of the nucleotides of the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. Such modifications are disclosed above in the context of "RNA modifications".

According to a further embodiment, the first nucleic acid molecule of the herein defined polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex can contain a lipid modification. Such a lipid-modified nucleic acid typically comprises a nucleic acid as defined herein. Such a lipid-modified first nucleic acid molecule of the polymeric carrier cargo complex or a lipid-modified second nucleic acid molecule administered in combination with the polymeric carrier cargo complex typically further comprises at least one linker covalently linked with that nucleic acid molecule, and at least one lipid covalently linked with the respective linker. Alternatively, the lipid-modified nucleic acid molecule comprises 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.

According to a further preferred embodiment, the at least one RNA of the inventive composition is complexed with lipids to form one or more liposomes, lipoplexes, or lipid nanoparticles. Therefore, in one embodiment, the inventive composition comprises liposomes, lipoplexes, and/or lipid nanoparticles comprising the at least one RNA.

Lipid-based formulations have been increasingly recognized as one of the most promising delivery systems for RNA due to their biocompatibility and their ease of large-scale production. Cationic lipids have been widely studied as synthetic materials for delivery of RNA. After mixing together, nucleic acids are condensed by cationic lipids to form lipid/nucleic acid complexes known as lipoplexes. These lipid complexes are able to protect genetic material from the action of nucleases and deliver it into cells by interacting with the negatively charged cell membrane. Lipoplexes can be prepared by directly mixing positively charged lipids at physiological pH with negatively charged nucleic acids.

Conventional liposomes consist of a lipid bilayer that can be composed of cationic, anionic, or neutral (phospho)lipids and cholesterol, which encloses an aqueous core. Both the lipid bilayer and the aqueous space can incorporate hydrophobic or hydrophilic compounds, respectively. Liposome

characteristics and behaviour in vivo can be modified by addition of a hydrophilic polymer coating, e.g. polyethylene glycol (PEG), to the liposome surface to confer steric stabilization. Furthermore, liposomes can be used for specific targeting by attaching ligands (e.g., antibodies, peptides, and carbohydrates) to its surface or to the terminal end of the attached PEG chains (Front Pharmacol. 2015 Dec 1;6:286).

Liposomes are colloidal lipid-based and surfactant-based delivery systems composed of a phospholipid bilayer surrounding an aqueous compartment. They may present as spherical vesicles and can range in size from 20 nm to a few microns. Cationic lipid-based liposomes are able to complex with negatively charged nucleic acids via electrostatic interactions, resulting in complexes that offer biocompatibility, low toxicity, and the possibility of the large-scale production required for in vivo clinical applications. Liposomes can fuse with the plasma membrane for uptake; once inside the cell, the liposomes are processed via the endocytic pathway and the genetic material is then released from the endosome/carrier into the cytoplasm. Liposomes have long been perceived as drug delivery vehicles because of their superior biocompatibility, given that liposomes are basically analogs of biological membranes, and can be prepared from both natural and synthetic phospholipids (Int J Nanomedicine. 2014; 9: 1833–1843).

Cationic liposomes have been traditionally the most commonly used non-viral delivery systems for oligonucleotides, including plasmid DNA, antisense oligos, and siRNA/small hairpin RNA-shRNA). Cationic lipids, such as DOTAP, (1,2-dioleoyl-3-trimethylammonium-propane) and DOTMA (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl-ammonium methyl sulfate) can form complexes or lipoplexes with negatively charged nucleic acids to form nanoparticles by electrostatic interaction, providing high in vitro transfection efficiency . Furthermore, neutral lipid-based nanoliposomes for RNA delivery as e.g. neutral 1,2-dioleoyl-sn-glycero-3- phosphatidylcholine (DOPC)-based nanoliposomes were developed. (Adv Drug Deliv Rev. 2014 Feb; 66: 110–116.).

Therefore, in one embodiment the at least one RNA of the inventive composition is complexed with cationic lipids and/or neutral lipids and thereby forms liposomes, lipid nanoparticles, lipoplexes or neutral lipid-based nanoliposomes.

Preferred cationic or polycationic compounds, which can be used as transfection or complexation agent may include cationic polysaccharides, for example chitosan, polybrene, cationic polymers, e.g. polyethyleneimine (PEI), cationic lipids, e.g. DOTMA: [1-(2,3-sioleyloxy)propyl)]-N,N,N-trimethylammonium chloride, DMRIE, di-C14-amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, DOPE: Dioleyl phosphatidylethanol-amine, DOSPA, DODAB, DOIC, DMEPC, DOGS: Dioctadecylamidoglicylspermin, DIMRI: Dimyristo-oxypropyl dimethyl hydroxyethyl ammonium bromide, DOTAP: dioleoyloxy-3-(trimethylammonio)propane, DC-6-14: O,O-ditetradecanoyl-N-(α-trimethylammonioacetyl)diethanolamine chloride, CLIP1: rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride, CLIP6: rac-[2(2,3-dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium, CLIP9: rac-[2(2,3-dihexadecyloxypropyl-oxysuccinyloxy)ethyl]-trimethylammonium, 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., polyimine(s), such as PEI: poly(ethyleneimine), poly(propyleneimine), etc., 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 from a cationic polymer as mentioned above) and of one or more hydrophilic or hydrophobic blocks (e.g. polyethyleneglycole); etc.

Additional pharmaceutically active compounds:

Furthermore the inventive composition may comprise at least one additional pharmaceutically active component/compound. Alternatively or in addition to that, the at least one additional pharmaceutically active component/compound may be co-administered concomitant to the composition according to the invention. Therefore, the at least one additional pharmaceutically active component/compound may be administered in combination with the at least one RNA of the inventive compostion or with the RNA containing composition according to the invention.

The phrases "administered in combination", co-administration or "concomitant administration" as used herein refers to a situation, where the inventive composition or an ingredient thereof is administered to a subject before, concomittantly or after the administration of a further pharmaceutically active component to the same subject. The time interval between the administration of the inventive composition or an ingredient thereof and the at least one second pharmaceutically active component depends on the nature and biological effect of the particular pharmaceutically active compononent and can be determined by a physician. Preferably the time interval is less than about 48 hours, more preferably less than about 24 hours, 12 hours, 6 hours, 4 hours, 2 hours, 1 hour, most preferably less than about 30 minutes, 15 minutes or 5 minutes. In a particularly preferred embodiment, the phrase "administered in combination" refers to concomitant administration of the inventive composition or an ingredient thereof and the at least one second pharmaceutically active component, i.e. the simultaneous administration of both compounds or the administration of both compounds within a time frame that typically comprises less than 5 minutes. The phrase "administered in combination" does not only refer to a situation, where the inventive composition or an ingredient thereof is in physical contact with the at least one second pharmaceutically active component or formulated together with said second pharmaceutically active component. The phrase "administered in combination" as used herein comprises also the separate administration of the inventive composition or an ingredient thereof and the second pharmaceutically active component (e.g. by two separate injections). Alternatively, the inventive composition or an ingredient thereof and the second pharmaceutically active component may be administered in combination by mixing the inventive composition or an ingredient thereof and the second pharmaceutically active component prior to administration and administering the mixture to a subject. When the inventive composition or an ingredient thereof is formulated together with the second pharmaceutically active component or when a composition as defined herein is used, the inventive composition or an ingredient thereof and the second pharmaceutically active component may further, independently from each other, administered in combination via any of the administration routes as described herein.

A pharmaceutically active component/compound in this connection is a compound that has a therapeutic effect to heal, ameliorate or prevent a particular indication or disease, namely a tumor or cancer disease. Such compounds include, without implying any limitation, peptides or proteins, preferably as defined herein, nucleic acids , preferably as defined herein, (therapeutically active)

low molecular weight organic or inorganic compounds (molecular weight less than 5000, preferably less than 1000), sugars, antigens or antibodies, preferably as defined herein, therapeutic agents already known in the prior art, antigenic cells, antigenic cellular fragments, cellular fractions, cell wall components (e.g. polysaccharides), modified, attenuated or de-activated (e.g. chemically or by irradiation) pathogens (virus, bacteria etc.), adjuvants, etc.

In a preferred embodiment, the inventive composition additionally comprises at least one further pharmaceutically active component/compound, wherein the at least one additional pharmaceutically active component is selected from cytokines, chemokines, suicide gene products, immunogenic proteins or peptides, apoptosis inducers, angiogenesis inhibitors, heat shock proteins, tumor antigens, β-catenin inhibitors, activators of the STING pathway, checkpoint modulators, innate immune activators, antibodies, dominant negative receptors and decoy receptors, inhibitors of myeloid derived suppressor cells (MDSCs), IDO pathway inhibitors, proteins or peptides that bind inhibitors of apoptosis, anti-bacterial agents, anti-viral agents, adjuvants, chemotherapeutic agents and kinase inhibitors.

Alternatively, or in addition to that, the at least one additional pharmaceutically active component may be co-administered concomitant to the at least one RNA of the RNA containing composition or the inventive composition or may be used in combination with the at least one RNA of the RNA containing composition or the inventive composition.

In this context protein-based cytokines, chemokines, suicide gene products, immunogenic proteins or peptides, apoptosis inducers, angiogenesis inhibitors, heat shock proteins, tumor antigens, β-catenin inhibitors, activators of the STING pathway, checkpoint modulators, innate immune activators, antibodies, dominant negative receptors and decoy receptors, inhibitors of myeloid derived suppressor cells (MDSCs), IDO pathway inhibitors, and proteins or peptides that bind inhibitors of apoptosis or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or fragments or variants thereof may be used as additional pharmaceutically active component.

1. Cytokines:

In this context protein-based cytokines, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or fragments or variants thereof may be used as additional pharmaceutically active component.

Preferably the cytokine is an interleukin (IL). One or more interleukins may be chosen e.g. from the following list: IL-1α, IL-1β, IL-1ra (antagonist), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10; IL-11, IL-12, IL-13, IL14, IL-15, IL-16, IL-17A, IL-17B, EL-17C, IL-17D, IL-17E, IL-17F, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28A/B, IL-29, IL-30, IL-31, IL-32, IL-33, IL-35. Moreover the cytokine may be one or more cytokines chosen from the TNF family, e.g. chosen from the following list: TNF, especially TNFα, LTα, LTβ, LIGHT, TWEAK, APRIL, BAFF, TL1A, GITRL, OX40L, CD40L (CD154), FASL, CD27L, CD30L, 4-1BBL, TRAIL, RANK ligand. Further examples of preferred cytokines may be chosen from the following list: FLT3 ligand, G-CSF, GM-CSF, IFNα/β/ω, IFNγ, LIF, M-CSF, MIF, OSM, Stem Cell Factor, TGFβ1, TGFβ2, TGFβ3, TSLP ligand.

Particularly preferred are cytokines chosen from the following list: IL-12, IL-15, IL-2, IFNγ, TNFα, IL-18, IFNα, IL-1β, IL-32, IL-7, IL-21, IL-8, GM-CSF.

In this context particularly preferred are cytokines as disclosed in Table 1 above.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072, and is combined with at least one cytokine as defined above, preferably IL-2, IL-12, CD40L or IL-15 or a fragment or variant thereof.

1. Chemokines

In this context protein-based chemokines, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or fragments or variants thereof may be used as additional pharmaceutically active component.

Preferred chemokines may be chosen from the following list: CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, XCL1, XCL2, CX3CL1.

In this context particularly preferred are chemokines as disclosed in Table 2 above.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one chemokine as defined above or a fragment or variant thereof.

2. Suicide enzymes

In this context protein-based suicide enzymes, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or fragments or variants thereof may be used as additional pharmaceutically active component.

The suicide enzyme is preferably a nucleotide metabolizing enzyme. Preferably the suicide enzyme is used in combination with a prodrug which is a substrate of the suicide enzyme, and which is converted to a cytotoxic compound by the suicide enzyme. One or more preferred suicide enzymes may be chosen from the following list: thymidine kinase, preferably a viral thymidine kinase, more preferrably Herpes simplex virus thymidine kinase, Varicella zoster thymidine kinase; a plant thymidine kinase, preferably a tomato thymidine kinase; cytosine deaminase, preferably bacterial cytosine deaminase or Yeast cytosine deaminase; deoxynucleoside kinase, preferably Drosophila melanogaster deoxynucleoside kinase; deoxycytidine kinase, preferably a mammalian deoxycytidine kinase, purine nucleoside phosphorylase, preferably a bacterial purine nucleoside phosphorylase.

In this context particularly preferred are suicide enzymes (suicide gene products) as disclosed in Table 3 and 4 above.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one suicide enzyme as defined above or a fragment or variant thereof.

3. Immunogenic proteins or peptides

In this context protein-based immunogenic proteins or peptides, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically

active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

The immunogenic protein or peptide is preferably a pathogenic antigen to utilize preexisting immunity against such antigens for treatment of tumor and/or cancer diseases. The memory immune response is triggered and the immune system is strengthened for attacking tumor cells.

Preferred examples of immunogenic proteins or peptides for this embodiment of the invention are proteins or peptides of widespread pathogens, i.e. pathogens with which every organism, in particular mammals, preferably humans, has a high probability of being infected at least once in his/her lifetime. These include, for example, any structural or non-structural protein or peptide of:

- influenza virus type A or B or any other orthomyxovirus (influenza type C),
- picornaviruses, such as rhinovirus or hepatitis A virus,
- togaviruses, such as alphavirus or rubivirus, e.g. Sindbis, Semliki-Forest or rubeolavirus (measles virus),
- rubella virus (German measles virus),
- coronaviruses, in particular subtypes HCV-229E or HCV-OC43,
- rhabdoviruses, such as rabies virus,
- paramyxoviruses, such as mumps virus,
- reoviruses, such as group A, B or C rotavirus,
- hepadnaviruses, such as hepatitis B virus,
- papoviruses, such as human papillomaviruses (HPV) of any serotype, especially from 1 to 75,
- adenoviruses, in particular type 1 to 47,
- herpesviruses, such as Herpes simplex virus 1, 2 or 3,
- cytomegalovirus (CMV), preferably CMVpp65,
- Epstein Barr virus (EBV),
- vaccinia viruses and
- the bacterium *Chlamydophila pneumoniae* (*Chlamydia pneumoniae*).

Further examples of preferred immunogenic proteins or peptides are proteins or peptides of pathogens which only seldom infect an organism. These proteins or peptide include, for example, any structural or non-structural protein or peptide of:

- Flaviviruses, such as dengue virus type 1 to 4, yellow fever virus, West Nile virus, Japanese encephalitis virus
- hepatitis C virus,
- caliciviruses,
- filoviruses, such as Ebola virus,
- bornaviruses,
- bunyaviruses, such as Rift Valley fever virus,
- arenaviruses, such as LCMV (lymphocytic choriomeningitis virus) or hemorrhagic fever viruses,
- retroviruses, such as HIV and
- parvoviruses.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one immunogenic protein or peptide as defined above, preferably influenza nucleoprotein (NP) or a fragment or variant thereof.

4. Apoptosis inducers:

In this context protein-based apoptosis inducers, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

Preferably, an apoptosis inducer is chosen from the group consisting of the Bcl-2 family and tumor suppressor protein p53 and ligands of transmembrane death receptors, especially the TNF (tumor necrosis factor) receptor gene superfamily, pro-apoptic receptor agonists and Beclin-1.

A particularily preferred apoptosis inducer in the context of the present invention is Beclin-1 (derived from the BECN1 gene).

Further preferred examples of apoptosis inducers may be chosen from the following list: Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, Blk, Cytochrome c, Caspases, especially Caspase 3, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Death domain, especially of Fas, preferably FasL, TNFα, Apo2L/TRAIL, agonist of DR4 and/or DR5, Apo3L, DR4 agonistic antibody, DR5 agonistic antibody, protein kinase R (PKR) (preferably constitutive active PKR), Granzyme B.

In this context particularly preferred are apoptosis inducers as disclosed in Table 5 and 6 above.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one apoptosis inducer as defined above, or a fragment or variant thereof.

5. Angiogenesis inhibitors

In this context protein-based angiogenesis inducers, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

Preferred examples of angiogenesis inhibitors according to the invention may be chosen from the following list: interferon alpha (IFN-α), (interferon beta) IFN-β, interferon gamma (IFN-γ), CXCL9, CXCL10, interleukin 12 (IL-12), platelet factor 4 (PF-4), tumor necrosis factor alpha (TNF-α), soluble fms-like tyrosine kinase 1 (sFLT-1), Fetal Liver Kinase 1 (FLK-1), Angiostatin, Endostatin, Vasostatin, Canstatin, Tumstatin, 16 kD prolacin fragment, tissue inhibitor of metalloproteinases 1 (TIMP-1), tissue inhibitor of metalloproteinases 2 (TIMP-2), tissue inhibitor of metalloproteinases 3 (TIMP-3), thrombospondin 1 (TSP-1), thrombospondin 2 (TSP-2), Maspin, PEX, soluble Tyrosine-protein kinase receptor 1 (sTie1), soluble Angiopoietin-1 receptor 2 (sTie2), Angiopoietin-1, Angiopoietin-2, Antivascular endothelial growth factor receptor 2 (VEGFR2) antibody (e.g. Alacizumab, Ramucirumab), Anti-vascular endothelial growth factor (VEGF) antibody (e.g. Brolucizumab, Ranibizumab, Bevacizumab), and Anti-vascular endothelial growth factor receptor 1 (VEGFR1) antibody (e.g. Icrucumab).

In this context particularly preferred are angiogenesis inhibitors as disclosed in Table 7 above.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one angiogenesis inhibitor as defined above, or a fragment or variant thereof.

6. Heat shock proteins:

In this context protein-based heat-shock proteins, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

Preferably, the heat shock protein may be chosen from the following list: HSP27, HSP47 (serpin H1), HSP60, HSP70, HSC70, GRP78 (BiP), HSP90, HSP110, GRP94 (gp96), GRP170 (ORP150), PDI/PDIA, CRT/CALR.

In this context particularly preferred are heat shock proteins as disclosed in Table 8 above.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one heat shock protein as defined above, or a fragment or variant thereof.

7. Tumour antigens:

In this context protein-based tumor antigens, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

In this context particularly preferred are tumor antignes as disclosed in Table 9 above.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one tumor antigen as defined above, or a fragment or variant thereof.

8. β-catenin inhibitors:

In this context protein-based β-catenin inhibitors, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

Particular preferred β-catenin inhibitors according to the present invention comprise TAT-NLS-BLBD-6, axin-1, TCF-4, GSK-3b, DKK-1, Dvl-1 derivatives or fragments thereof.

Chemical β-catenin inhibitors:

According to the present invention, the at least one additional active pharmaceutical ingredient which may be contained in the inventive composition, and/or which may be co-administered, or which may be combined with the inventive composition may be a chemical β-catenin inhibitors. Chemical β-catenin inhibitors are known in the art that may be administered according to the present invention. Preferably the chemical β-catenin inhibitor is chosen from the following list: PKF118-310, CGP049090, PKF115-584, PKF222-815, PKF118-744, ICG001, CCT036477, XAV939, acyl hydrazones (HQBA), molecules with 2,3,6-trisubstituted pyrido[2,3,-b]pyrazine core skeletons, carnosic acid, CCT031374, iCRT-3,5,14, NC043, Ibuprofin, aspirin.

The following table 13 summarizes examples of small molecular inhibitors of β-catenin signaling which are particularly preferred in this context.

Table 13: β-catenin inhibitors

Inhibitor	**Target**	**Reference**
PKF118-310, CGP049090, PKF115-584, PKF222-815 and PKF118-744	beta-catenin–TCF interaction	Lepourcelet et al., 2004. Cancer Cell 5:91–102
ICG001	beta-catenin–CBP interaction	Emami et al., 2004. Proc Natl Acad Sci USA 101:12682–7
CCT036477	beta-catenin–TCF interaction	Ewan et al., 2010. Cancer Res. 70:5963–73
XAV939	Tankyrase	Huang et al., 2009. Nature

Inhibitor	Target	Reference
		461:614–20
acyl hydrazones (HQBA)	Iron chelators	Song et al., 2011. Cancer Res. 71:7628–39; Coombs et al., 2012. Oncogene 31:213–25
molecules with 2,3,6-trisubstituted pyrido[2,3,-b] pyrazine core skeletons	beta-catenin	Gong et al., 2011. Bioorg Med Chem. 19:5639–47
carnosic acid	beta-catenin/BCL9	de la Roche et al., Nat Commun. 3:680
CCT031374	beta-catenin	Thorne et al., 2010. Nat Chem Biol. 6:829–36
iCRT-3,5,14, NC043	beta-catenin–TCF interaction	Wang et al., 2011. Cell Res. 21:730–40; Gonsalves et al., 2011. Proc Natl Acad Sci USA 108:5954–63
Ibuprofin, aspirin	Cox2 Inhibitors	Greenspan et al., 2011. Cancer Prev Res. 4:161–71

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one β-catenin inhibitor as defined above, or a fragment or variant thereof.

9. Activators of the STING pathway

In this context protein-based activators of the STING pathway, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component. Preferably, the at least one activator (stimulator) of the STING pathway is chosen from an activating protein or a constitutively active protein of the STING pathway, preferably DDX41, STING, cGAS, IRF3, TBK1 or STAT6 or a fragment or variant thereof.

Chemical STING-pathway activators:

In a further preferred embodiment the optional additional pharmaceutically active component may be selected from chemical activators of the STING pathway which are preferably selected from cyclic dinucleotides and xanthenone analogs.

Table 14 shows examples of chemical STING agonists. Further examples of STING agonists are disclosed in WO2014189805.

Table 14: Activators of STING pathway

Class of STING activator	**examples**
cyclic dinucleotides	3'3'-cGAMP, 2'3'-cGAMP, 2'2'-cGAMP, c-di-APM, c-di-GMP, c-di-IMP, c-di-UMP
xanthenone analogs	DMXAA, 5,6-dimethylxanthenone-4-acetic acid

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one STING pathway activator as defined above, or a fragment or variant thereof.

10. Checkpoint modulators

In this context protein-based checkpoint modulators, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

In preferred embodiments of the present invention the checkpoint modulator is a modulator of B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-H2, B7-H3, B7-H4, B7-H6, B7-H7/HHLA2, BTLA, CD28, CD28H/IGPR-1, CTLA-4, ICOS, PD-1, PD-L2/B7-DC, PDCD6, VISTA/B7-H5/PD-1H, BTN1A1/Butyrophilin, BTN2A1, BTN2A2/Butyrophilin 2A2, BTN3A1/2, BTN3A2, BTN3A3, BTNL2/Butyrophilin-like 2, BTNL3, BTNL4, BTNL6, BTNL8, BTNL9, BTNL10, CD277/BTN3A1, LAIR1, LAIR2, CD96, CD155/PVR, CRTAM, DNAM-1/CD226, Nectin-2/CD112, Nectin-3, TIGIT,

LILRA3/CD85e, LILRA4/CD85g/ILT7, LILRB1/CD85j/ILT2, LILRB2/CD85d/ILT4, LILRB3/CD85a/ILT5, LILRB4/CD85k/ILT3, 4-1BB/TNFRSF9/CD137, 4-1BB Ligand/TNFSF9, BAFF/BLyS/TNFSF13B, BAFF R/TNFRSF13C, CD27/TNFRSF7, CD27 Ligand/TNFSF7, CD30/TNFRSF8, CD30 Ligand/TNFSF8, CD40/TNFRSF5, CD40 Ligand/TNFSF5, DR3/TNFRSF25, GITR/TNFRSF18, GITR Ligand/TNFSF18, HVEM/TNFRSF14, LIGHT/TNFSF14, Lymphotoxin-alpha/TNF-beta, OX40/TNFRSF4, OX40 Ligand/TNFSF4, RELT/TNFRSF19L, TACI/TNFRSF13B, TL1A/TNFSF15, TNF-alpha, TNF RII/TNFRSF1B, 2B4/CD244/SLAMF4, BLAME/SLAMF8, CD2, CD2F-10/SLAMF9, CD48/SLAMF2, CD58/LFA-3, CD84/SLAMF5, CD229/SLAMF3, CRACC/SLAMF7, NTB-A/SLAMF6, SLAM/CD150, TIM-1/KIM-1/HAVCR, TIM-3, TIM-4, CD7, CD96, CD160, CD200, CD300a/LMIR1, CRTAM, DAP12, Dectin-1/CLEC7A, DPPIV/CD26, EphB6, Integrin alpha 4 beta 1, Integrin alpha 4 beta 7/LPAM-1, LAG-3, TIM-1/KIM-1/HAVCR, TIM-4, TSLP R, or any combinations thereof.

Preferably, the checkpoint modulator is selected from agonistic antibodies, antagonistic antibodies, ligands, dominant negative receptors and decoy receptors or combinations thereof.

Preferably, the agonistic antibody is chosen from the following list: anti-4-1BB, anti-OX40, anti-GITR, anti-CD28, anti-CD27, anti-CD-40anti-ICOS, anti-TNFRSF25, and anti-LIGHT.

Preferably, the antagonistic antibody is chosen from the list of anti-CTLA4, anti-PD1, anti-PD-L1, anti-Vista, anti-Tim-3, anti-LAG-3, and anti-BTLA.

Particularly preferred are the anti-CTLA-4 antibodies ipilimumab (Yervoy®), tremelimumab, and AGEN-1884.

Particularly preferred are the anti-PD1 antibodies Nivolumab (MDX-1106/BMS-936558/ONO-4538), (Brahmer et al., 2010. J Clin Oncol. 28(19):3167-75; PMID: 20516446); Pidilizumab (CT-011), (Berger et al., 2008. Clin Cancer Res. 14(10):3044-51; PMID: 18483370); Pembrolizumab (MK-3475, SCH 900475); AMP-224, and MEDI0680 (AMP-514).

Particularly preferred are the anti-PD-L1 antibodies MDX-1105/BMS-936559 (Brahmer et al. 2012. N Engl J Med. 366(26):2455-65; PMID: 22658128); atezolizumab (MPDL3280A/RG7446);durvalumab (MEDI4736); and avelumab (MSB0010718).

According to the present invention checkpoint modulators according to Table 15 are particularly preferred:

Table 15: Antibodies directed against immune checkpoint proteins

Name	Target
Urelumab	4-1BB/CD137
PF-05082566	4-1BB/CD137
8H9	B7-H3
Enoblituzumab	B7-H3
Ipilimumab	CD152/CTLA-4
Ticilimumab (= tremelimumab)	CD152/CTLA-4
Tremelimumab	CD152/CTLA-4
Varlilumab	CD27
Teneliximab	CD40
Vorsetuzumab mafodotin	CD70
Lirilumab	KIR2D
GSK-3174998	OX40
MEDI-6469	OX40
MEDI-6383	OX40
MEDI-0562	OX40
PF-04518600	OX40
RG-7888	OX40
PF-06801591	PD-1
BGBA-317	PD-1
MEDI-0680	PD-1
MK-3475	PD-1
Nivolumab	PD-1
PDR-001	PD-1
Pembrolizumab	PD-1
Pidilizumab	PD-1

REGN-2810	PD-1
SHR-1210	PD-1
TSR-042	PD-1
MDX-1106	PD-1
Merck 3745	PD-1
CT- 011	PD-1
MEDI-0680	PD-1
PDR001	PD-1
REGN2810	PD-1
BGB-108	PD-1
BGB-A317	PD-1
AMP-224	PD-1
Atezolizumab	PD-L1 (CD274)
Avelumab	PD-L1 (CD274)
BMS-936559	PD-L1 (CD274)
Durvalumab	PD-L1 (CD274)
MEDI-4736	PD-L1 (CD274)
MPDL33280A	PD-L1 (CD274)
YW243.55.S70	PD-L1 (CD274)
MDX-1105	PD-L1 (CD274)
MSB0010718C	PD-L1 (CD274)

In a further preferred embodiment the checkpoint modulator is a decoy receptor (e.g. a soluble receptor). Preferably, the decoy receptor is a soluble PD1 receptor. In a particularly preferred embodiment the RNA sequence encoding a soluble PD1 receptor comprises an RNA sequence according to SEQ ID NO: 389

In a further preferred embodiment the checkpoint modulator is a ligand of an immune checkpoint protein. Preferably, the ligand is CD40 Ligand (CD40L).

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one checkpoint modulator as defined above, preferably selected from an anti-CTLA4 antibody, an anti-PD1 antibody, an anti PD-L1 antibody, a CD40 ligand, or a soluble PD1 receptor, or a fragment or variant thereof.

11. Innate immune activators

In this context protein-based innate immune activators or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

In this context innate immune activators may be selected from mammalian, in particular human adjuvant proteins, which typically comprise any human protein or peptide, 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 adjuvant proteins are selected from the group consisting of proteins which are components and ligands of the signalling networks of the pattern recognition receptors including TLR, NLR and RLH, including TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11; NOD1, NOD2, NOD3, NOD4, NOD5, NALP1, NALP2, NALP3, NALP4, NALP5, NALP6, NALP6, NALP7, NALP7, NALP8, NALP9, NALP10, NALP11, NALP12, NALP13, NALP14,l IPAF, NAIP, CIITA, RIG-I, MDA5 and LGP2, the signal transducers of TLR signaling including adaptor proteins including e.g. Trif and Cardif; components of the Small-GTPases signalling (RhoA, Ras, Rac1, Cdc42, Rab etc.), components of the PIP signalling (PI3K, Src-Kinases, etc.), components of the MyD88-dependent signalling (MyD88, IRAK1, IRAK2, IRAK4, TIRAP, TRAF6 etc.), components of the MyD88-independent signalling (TICAM1, TICAM2, TRAF6, TBK1, IRF3, TAK1, IRAK1 etc.); the activated kinases including e.g. Akt, MEKK1, MKK1, MKK3, MKK4, MKK6, MKK7, ERK1, ERK2, GSK3, PKC kinases, PKD kinases, GSK3 kinases, JNK, p38MAPK, TAK1, IKK, and TAK1; the activated transcription factors including e.g. NF-κB, c-Fos, c-Jun, c-Myc, CREB, AP-1, Elk-1, ATF2, IRF-3, IRF-7.

Mammalian, in particular human adjuvant proteins may furthermore be selected from the group consisting of heat shock proteins, such as HSP10, HSP60, HSP65, HSP70, HSP75 and HSP90, gp96,

Fibrinogen, TypIII repeat extra domain A of fibronectin; or 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, or induced target genes including e.g. Beta-Defensin, cell surface proteins; or human adjuvant proteins including trif, flt-3 ligand, Gp96 or fibronectin, etc., or any species homolog of any of the above human adjuvant proteins. Furthermore HGMB1 may be used as adjuvant protein.

Mammalian, in particular human adjuvant proteins may furthermore comprise cytokines which induce or enhance an innate immune response, including IL-1 alpha, IL1 beta, IL-2, IL-6, IL-7, IL-8, IL-9, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, IL-21, IL-23, TNFalpha, IFNalpha, IFNbeta, IFNgamma, GM-CSF, G-CSF, M-CSF; chemokines including IL-8, IP-10, MCP-1, MIP-1alpha, RANTES, Eotaxin, CCL21; cytokines which are released from macrophages, including IL-1, IL-6, IL-8, IL-12 and TNF-alpha; as well as IL-1R1 and IL-1 alpha.

Therefore in this context it particularly preferred that the at least innate immune activator, is preferably an adjuvant protein, more preferably a human adjuvant protein, or a fragment or variant thereof.

In this context it is particularly preferred that I constitutive active variant of an adjuvant protein is used as innate immune activator, preferably a constitutive active variant of RIG-1 (ΔRIGI).

In another preferred embodiment the at least one innate immune activator is HGMB1, or a fragment or variant thereof.

In this context particularly preferred are innate immune activators as disclosed in Table 11 above.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one innate immune activator as defined above, or a fragment or variant thereof.

12. Antibodies, decoy receptors and dominant negative receptors

In this context protein-based antibodies, decoy receptors, or dominant negative recptors or fragments and variants thereof as disclosed above in the context of “coding RNA” may be used as

additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

According to the present invention antibodies according to Table 16 are particularly preferred:

Table 16: Antibodies directed against proteins accociated with tumor or cancer development

Name	**Target**
3F8	GD2
Abagovomab	CA-125 imitation
Abciximab	Platelet glycoprotein GPIIb/IIIa
Adecatumumab	EpCAM (CD326)
Afutuzumab	CD20
Alacizumab pegol	VEGFR2
Alemtuzumab	CD52
Altumomab pentetate	CEA
Amatuximab	mesothelin
Anatumomab mafenatox	5T4
Anetumab ravtansine	mesothelin
Apolizumab	HLA-DR beta
apomab	TRAIL-R2 (CD262)
Arcitumomab	CEA
Ascrinvacumab	ACVRL1
Bavituximab	phosphatidylserine
Bectumomab	CD22
Belimumab	BAFF
Besilesomab	CEA
Bevacizumab	VEGF-A
Bivatuzumab mertansine	CD44v6
Blinatumomab	CD19 x CD3
Brentuximab vedotin	CD30 (TNFRSF8)

Name	Target
Brontictuzumab	NOTCH1
canakinumab	IL-1β
Cantuzumab mertansine	CanAg
Cantuzumab ravtansine	MUC1 (CD227)
Capromab pendetide	PSMA
Carlumab	MCP-1
Catumaxomab	EpCAM x CD3
cBR-doxorubicin immunoconjugate	CD174 (Lewis Y)
Cetuximab	EGFR (HER1/ERBB1)
Citatuzumab bogatox	EpCAM
Cixutumumab	IGF-1R
Clivatuzumab tetraxetan	MUC1 (CD227)
Codrituzumab	glypican 3
Coltuximab ravtansine	CD19
Conatumumab	TRAIL-R2 (CD262)
Dacetuzumab	CD40
Dalotuzumab	IGF-1R
Dalotuzumab	insulin-like growth factor I receptor
Daratumumab	CD38 (cyclic ADP ribose hydrolase)
Demcizumab	DLL4
Denintuzumab mafodotin	CD19
Denosumab	RANKL
Depatuxizumab	EGFR (HER1/ERBB1)
Derlotuximab	histone complex
Detumomab	unknown (B-lymphoma cells)
Dinutuximab	B4GALNT1
Drozitumab	TRAIL-R2 (CD262)
Duligotumab	HER3 (ERBB3)
Duligotuzumab	EGFR (HER1/ERBB1)

Name	Target
Dusigitumab	ILGF2
Ecromeximab	GD3 ganglioside
Edrecolomab	EpCAM
Elgemtumab	ERBB3
Elotuzumab	SLAMF7 (CD319)
Elsilimomab	IL-6
Emactuzumab	CSF1R
Emibetuzumab	HGFR
Emibetuzumab	MET
Enavatuzumab	TNFRSF12A
Enfortumab vedotin	AGS-22M6
Enoticumab	DLL4
Ensituximab	MUC5AC
Epitumomab cituxetan	MUC1 (CD227)
Epratuzumab	CD22
Ertumaxomab	HER2 (ERBB2/neu) x CD3
Etaracizumab	integrin α5β3
Faralimomab	IFNA1
Farletuzumab	FOLR1 alpha
FBTA	CD20 x CD3
Ficlatuzumab	HGFR
Figitumumab	IGF-1R
Flanvotumab	TYRP1(glycoprotein 75)
Fresolimumab	TGF-β
Futuximab	EGFR (HER1/ERBB1)
Galiximab	CD80
Gantiumab	IGF-1R
Gemtuzumab ozogamicin	CD33
Girentuximab	Carbonic anhydrase 9 (CA9/CAIX)

Name	Target
Glembatumumab vedotin	GPNMB
glycooptimized trastuzumab-GEX	HER2 (ERBB2/neu)
Ibritumomab tiuxetan	CD20
Icrucumab	VEGFR-1
Igovomab	MUC16
IMAB362	Claudin-18 (CLDN18.2)
Imgatuzumab	EGFR (HER1/ERBB1)
Indatuximab ravtansine	SDC1
Indusatumab vedotin	GUCY2C
inebilizumab	CD19
Inotuzumab ozogamicin	CD22
Intetumumab	CD51
Iratumumab	CD30 (TNFRSF8)
Isatuximab	CD38
Labetuzumab	CEA
Lenzilumab	CSF2
Lexatumumab	TRAIL-R2 (CD262)
Lifastuzumab vedotin	NaPi2B
Lilotomab satetraxetan	CD37
Lintuzumab	CD33
Lorvotuzumab mertansine	CD56
Lucatumumab	CD40
Lumiliximab	CD23 (IgE receptor)
Lumretuzumab	ERBB3
Mapatumumab	TRAIL-R1 (CD261)
Margetuximab	HER2 (ERBB2/neu)
Matuzumab	EGFR (HER1/ERBB1)
Mepolizumab	IL-5
Milatuzumab	CD74

Name	Target
Minretumomab	TAG-72
Mirvetuximab soravtansine	FOLR1 alpha
Mitumomab	GD3 (ganglioside)
Mogamulizumab	CCR4
Moxetumomab pasudotox	CD22
Nacolomab tafenatox	C242 antigen
Naptumomab estafenatox	5T4
Narnatumab	RON
Necitumumab	EGFR (HER1/ERBB1)
Nesvacumab	ANGPT2 (angiopoietin 2)
Nimotuzumab	EGFR (HER1/ERBB1)
Nofetumomab merpentan	EpCAM
binutuzumab	CD20
Ocaratuzumab	CD20
Ofatumumab	CD20
Olaratumab	PDGFRα
Onartuzumab	MET
Ontuxizumab	CD248 (TEM1)
Oportuzumab monatox	EpCAM
Oregovomab	CA-125
Otlertuzumab	CD37
Panitumumab	EGFR (HER1/ERBB1)
Pankomab	MUC1 (tumor specific glycosylation)
Parsatuzumab	EGFL7
Pasotuxizumab	FOLH1
Patritumab	HER3 (ERBB3)
Pemtumomab	MUC1 (CD227)
Pertuzumab	HER2 (ERBB2/neu)
Pinatuzumab vedotin	CD22

Name	Target
Pintumomab	adenocarcinoma antigen
Polatuzumab vedotin	CD79B
Racotumomab	NGcGM3
Radretumab	EDB (fibronectin extra domain-B)
Ramucirumab	VEGFR2
Rilotumumab	HGFR
Rituximab	CD20
Robatumumab	IGF-1R
Sacituzumab govitecan	Trop-2 (tumor-associated calcium signal transducer 2/EGP-1)
Samalizumab	CD200 (OX-2 membrane glycoprotein)
Satumomab pendetide	TAG-72
Seribantumab	ERBB3
Seribantumab	HER3 (ERBB3)
SGN-CDA	CD19
SGN-CDA	CD33
Sibrotuzumab	FAP
Siltuximab	IL-6
Simtuzumab	LOXL2
Sofituzumab vedotin	CA 125
Solitomab	EpCAM
Sonepcizumab	S1P (sphingosine-1-phosphate)
Tacatuzumab tetraxetan	AFP (alpha-fetoprotein)
Taplitumomab paptox	CD19
Tarextumab	Notch receptor
Tenatumomab	TN-C (tenascin C)
Teprotumumab	CD221
Tetulomab	CD37
TGN	CD28

Name	Target
Tigatuzumab	TRAIL-R2 (CD262)
Lebrikizumab	IL-13
Tocilizumab	IL-6R
Tositumomab	CD20
Tovetumab	CD140a
Tovetumab	PDGFRα
Trastuzumab	HER2 (ERBB2/neu)
Trastuzumab emtansine	HER2 (ERBB2/neu)
TRBS	GD2
Tucotuzumab celmoleukin	EpCAM
ublituximab	CD20
Ublituximab	MS4A1
Ulocuplumab	CXCR4
Vandortuzumab vedotin	STEAP1
Vantictumab	FZD7
Vanucizumab	Ang-2 (angiopoietin 2) x VEGF-A
Veltuzumab	CD20
Vesencumab	NRP1
Volociximab	integrin α5β1
Votumumab	CTAA16.88
Zalutumumab	EGFR (HER1/ERBB1)
Zanolimumab	CD4
Zatuximab	HER1 (EGFR/ERBB1)

Preferably, the neutralizing antibody is chosen from the list of anti-IL-10 and anti-TGFbeta.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one antibody, preferably anti-CD73 antibody or at least one decoy receptor as defined above, or a fragment or variant thereof.

Furthermore, the at least one antibody may preferably chosen from anti-CD73 antibodies or fragments or variants thereof.

In a further particularly preferred embodiment the at least one antibody is chosen from an antibody directed against CCR5/CD195 or from an antibody directed against its ligand CCL5/RANTES or fragments or variants thereof.

In a particularly preferred embodiment the decoy receptor is a soluble CCR5 (chemokine receptor type 5, also known as CD195).

In a particularly preferred embodiment the dominant negative receptor is dominant negative CCR5 (chemokine receptor type 5, also known as CD195).

13. Inhibitors of myeloid derived suppressor cells (MDSCs)

In this context protein-based inhibitors of myeloid derived suppressor cells (MDSCs), or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

It is particularly preferred to use anti IL-17 antibodies and IL-12 as inhibitors of MDSCs.

In the context of the invention, MDSC inhibition can be achieved by direct deactivation of MDSCs (e.g., chemical NO inhibitors (PDE-5 inhibitors, NO-aspirins, L-NAME), Arginase inhibitors (PDE-5 inhibitors, COX2 inhibitors, NOHA, L-NAME), ROS inhibitors(synthetic Triterpenoids)), by blocking differentiation of MDSCs into mature cells (e.g., ATRA, Vitamin A, Vitamin D3, CpG), by blocking the cell development of MDSCs (e.g. bisphosphorates (zolodronic acid), modulators of cell signaling (JAK2/STAT3 inhibitors, Multi-Kinase inhibitors, VEGF inhibitors)), or by depletion of MDSCs (e.g., cytotoxic agents (gemcitabine, cisplatin, paclitaxel, 5-fluorouracil) or HSP 90 inhibitors (17-DMAG)). Therefore these compounds may also be used as additional pharmaceutically active compound.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one inhibitor of MDSCs as defined above, or a fragment or variant thereof.

14. IDO pathway inhibitors

In this context protein-based IDO pathway inhibitors, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

Chemical IDO pathway inhibitor:

In a further preferred embodiment the additional pharmaceutically active component may be selected from an IDO pathway inhibitor, which is preferably selected from small molecule inhibitor. Preferably the IDO pathway inhibitor is chosen from the following list: Indoximod (the D isomer of 1-methyl-tryptophan) and NLG919).

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one IDO pathway inhibitor as defined above, or a fragment or variant thereof.

15. Proteins or peptides that bind inhibitors of apoptosis

Apoptosis is a tightly regulated cellular process and faulty regulation of apoptosis is a hallmark of human cancers. Targeting key apoptosis regulators with the goal to restore apoptosis in tumor cells has been pursued as a new cancer therapeutic strategy. XIAP, cIAP1, and cIAP2, members of inhibitor of apoptosis (IAP) proteins, are critical regulators of cell death and survival and are attractive targets for new cancer therapy. The SMAC/DIABLO protein is an endogenous antagonist of XIAP, cIAP1, and cIAP2. In the last decade, intense research efforts have resulted in the design and development of several small-molecule SMAC mimetics now in clinical trials for cancer treatment.

In a further preferred embodiment, the inventive composition comprises at least one molecule that binds inhibitors of apoptosis proteins (IAPs) and thus sensitize cancer cells to apoptotic death.

Therefore it is particularly preferred that the the inventive RNA containing composition comprises at least one molecule that binds inhibitors of apoptosis, such as SMAC mimetics.

Particularly preferred proteins or peptides that bind IAPs according to the present invention comprise Omi/HtrA2, Smac, Smac derived peptides, Smac/DIABLO, and XAF1 (XIAP-associated factor 1) and fragments or variants thereof.

In this context proteins or peptides that bind inhibitors of apoptosis, or fragments and variants thereof as disclosed above in the context of "coding RNA" may be used as additional pharmaceutically active component. Alternatively, nucleic acids encoding these proteins or peptides or fragments or variants thereof may be used as additional pharmaceutically active component.

Therefore it is particularly preferred that the additional pharmaceutically active component is selected from proteins or peptides that bind inhibitors of apoptosis, such as SMAC mimetics. Furthermore it is particularly preferred that such SMAC mimetics used as additional pharmaceutically active component are small molecules inhibiting inhibitors of apoptosis.

In a particularly preferred embodiment, the at least one RNA of the RNA containing composition is an immunostimulating RNA, preferably according to SEQ ID NOs. 5, 394, or 10072 and is combined with at least one proteins or peptides that bind inhibitors of apoptosis as defined above, or a fragment or variant thereof.

16. Anti-bacterial agent:

According to the present invention, the at least one additional pharmaceutically active component which may be contained in the inventive composition, and/or which may be co-administered, may be an anti-bacterial agent. In this context, any anti-bacterial agents known to one of skill in the art may be used in combination with the components of the inventive composition as defined herein. Non-limiting examples of anti-bacterial agents include Amikacin, Amoxicillin, Amoxicillin-clavulanic acid, Amphothericin-B, Ampicillin, Ampicllin-sulbactam, Apramycin, Azithromycin, Aztreonam, Bacitracin, Benzylpenicillin, Caspofungin, Cefaclor, Cefadroxil, Cefalexin, Cefalothin, Cefazolin, Cefdinir, Cefepime, Cefixime, Cefmenoxime, Cefoperazone, Cefoperazone-sulbactam, Cefotaxime,

Cefoxitin, Cefbirome, Cefpodoxime, Cefpodoxime-clavulanic acid, Cefpodoxime-sulbactam, Cefbrozil, Cefquinome, Ceftazidime, Ceftibutin, Ceftiofur, Ceftobiprole, Ceftriaxon, Cefuroxime, Chloramphenicole, Florfenicole, Ciprofloxacin, Clarithromycin, Clinafloxacin, Clindamycin, Cloxacillin, Colistin, Cotrimoxazol (Trimthoprim/sulphamethoxazole), Dalbavancin, Dalfopristin/Quinopristin, Daptomycin, Dibekacin, Dicloxacillin, Doripenem, Doxycycline, Enrofloxacin, Ertapenem, Erythromycin, Flucloxacillin, Fluconazol, Flucytosin, Fosfomycin, Fusidic acid, Garenoxacin, Gatifloxacin, Gemifloxacin, Gentamicin, Imipenem, Itraconazole, Kanamycin, Ketoconazole, Levofloxacin, Lincomycin, Linezolid, Loracarbef, Mecillnam (amdinocillin), Meropenem, Metronidazole, Meziocillin, Mezlocillin- sulbactam, Minocycline, Moxifloxacin, Mupirocin, Nalidixic acid, Neomycin, Netilmicin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Pefloxacin, Penicillin V, Piperacillin, Piperacillin-sulbactam, Piperacillin-tazobactam, Rifampicin, Roxythromycin, Sparfloxacin, Spectinomycin, Spiramycin, Streptomycin, Sulbactam, Sulfamethoxazole, Teicoplanin, Telavancin, Telithromycin, Temocillin, Tetracyklin, Ticarcillin, Ticarcillin-clavulanic acid, Tigecycline, Tobramycin, Trimethoprim, Trovafloxacin, Tylosin, Vancomycin, Virginiamycin, and Voriconazole.

17. Anti-viral agents:

According to the present invention, the at least one additional pharmaceutically active component/compound, which may be contained in the inventive composition, and/or which may be co-administered, may be an anti-viral agents, preferably, but are not limited to, nucleoside analogs (e.g., zidovudine, acyclovir, gangcyclovir, vidarabine, idoxuridine, trifluridine, and ribavirin), foscarnet, amantadine, peramivir, rimantadine, saquinavir, indinavir, ritonavir, alpha-interferons and other interferons, AZT, t-705, zanamivir (Relenza®), and oseltamivir (Tamiflu®). Other anti-viral agents include influenza virus vaccines, e.g., Fluarix® (Glaxo SmithKline), FluMist® (MedImmune Vaccines), Fluvirin® (Chiron Corporation), Flulaval® (GlaxoSmithKline), Afluria® (CSL Biotherapies Inc.), Agriflu® (Novartis) or Fluzone® (Aventis Pasteur).

18. Drugs:

In some embodiments, the additional pharmaceutically active component/compound may include at least one drug. The term “drug” is intended to include any substance that, when introduced or absorbed into the body of a living organism, alters normal bodily or cellular function. Some non-limiting examples of suitable drugs, including combinations and alternative forms of the drugs such

as alternative salt forms, free acid form, free base forms, pro-drugs and hydrates, include: analgesics/antipyretics (e.g., aspirin, acetaminophen, ibuprofen, naproxen sodium, buprenorphine, propoxyphene hydrochloride, propoxyphene napsylate, meperidine hydrochloride, hydromorphone hydrochloride, morphine, oxycodone, codeine, dihydrocodeine bitartrate, pentazocine, hydrocodone bitartrate, levorphanol, diflunisal, trolamine salicylate, nalbuphine hydrochloride, mefenamic acid, butorphanol, choline salicylate, butalbital, phenyltoloxamine citrate, diphenhydramine citrate, methotrimeprazine, cinnamedrine hydrochloride, and meprobamate); antiasthmatics (e.g., ketotifen and traxanox); antibiotics (e.g., neomycin, streptomycin, chloramphenicol, cephalosporin, ampicillin, penicillin, tetracycline, and ciprofloxacin); antidepressants (e.g., nefopam, oxypertine, doxepin, amoxapine, trazodone, amitriptyline, maprotiline, phenelzine, desipramine, nortriptyline, tranylcypromine, fluoxetine, imipramine, imipramine pamoate, isocarboxazid, trimipramine, and protriptyline); antidiabetics (e.g., biguanides and sulfonylurea derivatives); antifungal agents (e.g., griseofulvin, ketoconazole, itraconazole, amphotericin B, nystatin, and candicidin); antihypertensive agents (e.g., propanolol, propafenone, oxyprenolol, nifedipine, reserpine, trimethaphan, phenoxybenzamine, pargyline hydrochloride, deserpidine, diazoxide, guanethidine monosulfate, minoxidil, rescinnamine, sodium nitroprusside, rauwolfia serpentine, alseroxylon, and phentolamine); anti-inflammatories (e.g., (non-steroidal) indomethacin, ketoprofen, flurbiprofen, naproxen, ibuprofen, ramifenazone, piroxicam, (steroidal) cortisone, dexamethasone, fluazacort, deflazacort, celecoxib, rofecoxib, hydrocortisone, prednisolone, and prednisone); antineoplastics (e.g., cyclophosphamide, actinomycin, bleomycin, dactinomycin, daunorubicin, doxorubicin, epirubicin, mitomycin, methotrexate, fluorouracil, gemcitabine, carboplatin, carmustine (BCNU), methyl-CCNU, cisplatin, etoposide, camptothecin and derivatives thereof, phenesterine, paclitaxel and derivatives thereof, docetaxel and derivatives thereof, vinblastine, vincristine, goserelin, leuprolide, tamoxifen, interferon alfa, retinoic acid (ATRA), nitrogen mustard alkylating agents, and piposulfan); antianxiety agents (e.g., lorazepam, buspirone, prazepam, chlordiazepoxide, oxazepam, clorazepate dipotassium, diazepam, hydroxyzine pamoate, hydroxyzine hydrochloride, alprazolam, droperidol, halazepam, chlormezanone, and dantrolene); immunosuppressive agents (e.g., cyclosporine, azathioprine, mizoribine, and FK506 (tacrolimus)); antimigraine agents (e.g., ergotamine, propanolol, isometheptene mucate, and dichloralphenazone); sedatives/hypnotics (e.g., barbiturates such as pentobarbital, pentobarbital, and secobarbital; and benzodiazapines such as flurazepam hydrochloride, triazolam, and midazolam); antianginal agents (e.g., beta-adrenergic

blockers; calcium channel blockers such as nifedipine, and diltiazem; and nitrates such as nitroglycerin, isosorbide dinitrate, pentearythritol tetranitrate, and erythrityl tetranitrate); antipsychotic agents (e.g., haloperidol, loxapine succinate, loxapine hydrochloride, thioridazine, thioridazine hydrochloride, thiothixene, fluphenazine, fluphenazine decanoate, fluphenazine enanthate, trifluoperazine, chlorpromazine, perphenazine, lithium citrate, and prochlorperazine); antimanic agents (e.g., lithium carbonate); antiarrhythmics (e.g., bretylium tosylate, esmolol, verapamil, amiodarone, encamide, digoxin, digitoxin, mexiletine, disopyramide phosphate, procainamide, quinidine sulfate, quinidine gluconate, quinidine polygalacturonate, flecamide acetate, tocamide, and lidocaine); antiarthritic agents (e.g., phenylbutazone, sulindac, penicillanine, salsalate, piroxicam, azathioprine, indomethacin, meclofenamate, gold sodium thiomalate, ketoprofen, auranofin, aurothioglucose, and tolmetin sodium); antigout agents (e.g., colchicine, and allopurinol); anticoagulants (e.g., heparin, heparin sodium, and warfarin sodium); thrombolytic agents (e.g., urokinase, streptokinase, and alteplase); antifibrinolytic agents (e.g., aminocaproic acid); hemorheologic agents (e.g., pentoxifylline); antiplatelet agents (e.g., aspirin); anticonvulsants (e.g., valproic acid, divalproex sodium, phenyloin, phenyloin sodium, clonazepam, primidone, phenobarbital, carbamazepine, amobarbital sodium, methsuximide, metharbital, mephobarbital, mephenyloin, phensuximide, paramethadione, ethotoin, phenacemide, secobarbital sodium, clorazepate dipotassium, and trimethadione); antiparkinson agents (e.g., ethosuximide); antihistamines/antipruritics (e.g., hydroxyzine, diphenhydramine, chlorpheniramine, brompheniramine maleate, cyproheptadine hydrochloride, terfenadine, clemastine fumarate, triprolidine, carbinoxamine, diphenylpyraline, phenindamine, azatadine, tripelennamine, dexchlorpheniramine maleate, methdilazine, and); agents useful for calcium regulation (e.g., calcitonin, and parathyroid hormone); antibacterial agents (e.g., amikacin sulfate, aztreonam, chloramphenicol, chloramphenicol palmitate, ciprofloxacin, clindamycin, clindamycin palmitate, clindamycin phosphate, metronidazole, metronidazole hydrochloride, gentamicin sulfate, lincomycin hydrochloride, tobramycin sulfate, vancomycin hydrochloride, polymyxin B sulfate, colistimethate sodium, and colistin sulfate); antiviral agents (e.g., interferon alpha, beta or gamma, zidovudine, amantadine hydrochloride, ribavirin, and acyclovir); antimicrobials (e.g., cephalosporins such as cefazolin sodium, cephradine, cefaclor, cephapirin sodium, ceftizoxime sodium, cefoperazone sodium, cefotetan disodium, cefuroxime axetil, cefotaxime sodium, cefadroxil monohydrate, cephalexin, cephalothin sodium, cephalexin hydrochloride monohydrate, cefamandole nafate, cefoxitin sodium, cefonicid sodium, ceforanide, ceftriaxone sodium,

ceftazidime, cefadroxil, cephradine, and cefuroxime sodium; penicillins such as ampicillin, amoxicillin, penicillin G benzathine, cyclacillin, ampicillin sodium, penicillin G potassium, penicillin V potassium, piperacillin sodium, oxacillin sodium, bacampicillin hydrochloride, cloxacillin sodium, ticarcillin disodium, azlocillin sodium, carbenicillin indanyl sodium, penicillin G procaine, methicillin sodium, and nafcillin sodium; macrolides such as, azithromycin, clarithromycin, and erythromycins such as erythromycin ethylsuccinate, erythromycin, erythromycin estolate, erythromycin lactobionate, erythromycin stearate, and erythromycin ethylsuccinate; and tetracyclines such as tetracycline hydrochloride, doxycycline hyclate, and minocycline hydrochloride); anti-infectives (e.g., GM-CSF); bronchodilators (e.g., sympathomimetics such as epinephrine hydrochloride, metaproterenol sulfate, terbutaline sulfate, isoetharine, isoetharine mesylate, isoetharine hydrochloride, albuterol sulfate, albuterol, bitolterolmesylate, isoproterenol hydrochloride, terbutaline sulfate, epinephrine bitartrate, metaproterenol sulfate, epinephrine, and epinephrine bitartrate; anticholinergic agents such as ipratropium bromide; xanthines such as aminophylline, dyphylline, metaproterenol sulfate, and theophylline; mast cell stabilizers such as cromolyn sodium; inhalant corticosteroids such as beclomethasone dipropionate (BDP), and beclomethasone dipropionate monohydrate; salbutamol; ipratropium bromide; budesonide; salmeterol; xinafoate; triamcinolone; nedocromil sodium; flunisolide; fluticasone propionate; steroidal compounds and hormones (e.g., androgens such as danazol, testosterone cypionate, fluoxymesterone, ethyltestosterone, testosterone enathate, methyltestosterone; estrogens such as estradiol, estropipate, and conjugated estrogens; progestins such as methoxyprogesterone acetate, and norethindrone acetate; corticosteroids such as triamcinolone, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, dexamethasone acetate, prednisone, methylprednisolone acetate suspension, triamcinolone acetonide, methylprednisolone, prednisolone sodium phosphate, methylprednisolone sodium succinate, hydrocortisone sodium succinate, triamcinolone hexacetonide, hydrocortisone, hydrocortisone cypionate, prednisolone, fludrocortisone acetate, paramethasone acetate, prednisolone tebutate, prednisolone acetate, prednisolone sodium phosphate, and thyroid hormones such as levothyroxine sodium); hypoglycemic agents (e.g., human insulin, purified beef insulin, purified pork insulin, glyburide, metformin, chlorpropamide, glipizide, tolbutamide, and tolazamide); hypolipidemic agents (e.g., clofibrate, dextrothyroxine sodium, probucol, pravastitin, atorvastatin, lovastatin, and niacin); proteins (e.g., DNase, alginase, superoxide dismutase, and lipase); nucleic acids (e.g., anti-sense nucleic acids); agents useful for erythropoiesis stimulation (e.g.,

erythropoietin); antiulcer/antireflux agents (e.g., famotidine, cimetidine, and ranitidine hydrochloride); antinauseants/antiemetics (e.g., meclizine hydrochloride, nabilone, prochlorperazine, dimenhydrinate, promethazine hydrochloride, thiethylperazine, and scopolamine); as well as other drugs useful in the compositions and methods described herein include mitotane, halonitrosoureas, anthrocyclines, ellipticine, ceftriaxone, ketoconazole, ceftazidime, oxaprozin, valacyclovir, urofollitropin, famciclovir, flutamide, enalapril, itraconazole, buspirone, gabapentin, fosinopril, tramadol, acarbose, lorazepam, follitropin, omeprazole, fluoxetine, lisinopril, tramadol, levofloxacin, zafirlukast, interferon, growth hormone, interleukin, erythropoietin, granulocyte stimulating factor, nizatidine, bupropion, perindopril, erbumine, adenosine, alendronate, alprostadil, benazepril, betaxolol, bleomycin sulfate, dexfenfluramine, diltiazem, fentanyl, flecamide, gemcitabine, glatiramer acetate, granisetron, lamivudine, mangafodipir trisodium, mesalamine, metoprolol fumarate, metronidazole, miglitol, moexipril, monteleukast, octreotide acetate, olopatadine, paricalcitol, somatropin, sumatriptan succinate, tacrine, verapamil, nabumetone, trovafloxacin, dolasetron, zidovudine, finasteride, tobramycin, isradipine, tolcapone, enoxaparin, fluconazole, lansoprazole, terbinafine, pamidronate, didanosine, diclofenac, cisapride, venlafaxine, troglitazone, fluvastatin, losartan, imiglucerase, donepezil, olanzapine, valsartan, fexofenadine, calcitonin, and ipratropium bromide. In some embodiments, the drug may be water soluble. In some embodiments, the drug may not be water soluble

19. Combination with standard therapy

According to the present invention, the at least one additional pharmaceutically active component/compound which may be contained in the inventive composition, and/or which may be co-administered, may be selected from any standard therapy used for the treatment of the particular tumor or cancer disease, e.g any chemotherapy, checkpoint modulator, kinase inhibitor etc.

Adjuvants and further components:

According to the present invention, the at least one additional pharmaceutically active component/compound which may be contained in the inventive composition, and/or which may be co-administered may be an adjuvant. According to a specific embodiment, the inventive 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 composition preferably elicits an innate immune response due to the adjuvant, optionally contained therein. Preferably, such an adjuvant may be selected from an 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.

Particularly preferred as adjuvants suitable for depot and delivery are cationic or polycationic compounds as defined above for the RNA of the inventive composition as vehicle, transfection or complexation agent.

Furthermore, the inventive composition may comprise one or more additional adjuvants which are suitable to initiate or increase an immune response of the innate immune system, i.e. a non-specific immune response, particularly by binding to pathogen-associated molecular patterns (PAMPs). With other words, when administered, the pharmaceutical composition preferably elicits an innate immune response due to the adjuvant, optionally contained therein. Preferably, such an adjuvant may be selected from an 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.

Also 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 and/or suitable for depot and delivery of the components of the inventive composition. Preferred as adjuvants suitable for depot and delivery are cationic or polycationic compounds as defined above. Likewise, the adjuvant may be selected from the group consisting of, e.g., cationic or polycationic compounds as defined above, from chitosan, TDM, MDP, muramyl dipeptide, pluronics, alum solution, aluminium hydroxide, ADJUMERTM (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); AVRIDINETM (propanediamine); BAY R1005TM ((N-(2-deoxy-2-L-leucylaminob- D-glucopyranosyl)-N-octadecyl-dodecanoyl-amide hydroacetate); CALCITRIOLTM (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-D35 glutamine (GMDP), ii) dimethyldioctadecylammonium chloride (DDA), iii) zinc-L-proline salt complex (ZnPro-8); GM-CSF); GMDP (N-acetylglucosaminyl-(b1-4)-N-acetylmuramyl-L47 alanyl-D-isoglutamine); imiquimod (1-(2-methypropyl)-1H-imidazo[4,5-c]quinoline-4-amine); ImmTherTM (N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-glycerol dipalmitate); DRVs (immunoliposomes prepared from dehydration-rehydration vesicles); interferongamma; interleukin-1beta; interleukin-2; interleukin-7; interleukin-12; ISCOMSTM; ISCOPREP 7.0.3. TM; liposomes; LOXORIBINETM (7-allyl-8-oxoguanosine); LT 5 oral adjuvant (E.coli labile enterotoxin-protoxin); microspheres and microparticles of any composition; MF59TM; (squalenewater emulsion); MONTANIDE ISA 51TM (purified incomplete Freund's adjuvant); MONTANIDE ISA 720TM (metabolisable oil adjuvant); MPLTM (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); MURAMETIDETM (Nac-Mur-L-Ala-D-Gln-OCH3); MURAPALMITINETM and DMURAPALMITINETM (Nac-Mur-L-Thr-D-isoGln-sn-glyceroldipalmitoyl); NAGO (neuraminidase- galactose oxidase); nanospheres or nanoparticles of any composition; NISVs (non-ionic surfactant vesicles); PLEURANTM (β-glucan); PLGA, PGA and PLA (homo- and co-polymers of lactic acid and glycolic acid; microspheres/nanospheres); PLURONIC L121TM; PMMA (polymethylmethacrylate); PODDSTM (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); STIMULONTM (QS-21); Quil-A (Quil-A saponin); S-28463 (4-amino-otec-dimethyl-2-ethoxymethyl-1H-imidazo[4,5-c]quinoline-1-ethanol); SAF-1TM ("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-Aladipalmitoxypropylamide); Theronyl-MDP (TermurtideTM or [thr 1]-MDP; N-acetylmuramyl-Lthreonyl-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, 35 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.

Particularly preferred, an adjuvant may be selected from adjuvants, which support induction of a Th1-immune response or maturation of naïve T-cells, such as GM-CSF, IL-12, IFNg, any RNA as defined herein, preferably an immunostimulatory RNA, CpG DNA, etc.

It is possible that the inventive composition contains besides the at least one RNA as described above further components which are selected from the group comprising: further antigens or further antigen-providing nucleic acids; a further immunotherapeutic agent; one or more auxiliary substances; or any further compound, which is known to be immunostimulating due to its binding affinity (as ligands) to human Toll-like receptors; and/or an adjuvant nucleic acid, preferably an immunostimulatory RNA (isRNA).

The inventive composition can additionally contain one or more auxiliary substances in order to increase its immunogenicity or immunostimulatory capacity, if desired. A synergistic action of the at least one RNA as defined herein and of an auxiliary substance, which may be optionally contained in the inventive composition, 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 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, IFN-alpha, IFN-beta, IFN-gamma, GM-CSF, G-CSF, M-CSF, LT-beta or TNF-alpha, growth factors, such as hGH.

The inventive composition may contain any further compound, which is known to be immunostimulating 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.

Further additives which may be included in the inventive composition 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.

Pharmaceutical composition:

In a further aspect, the present invention also provides a pharmaceutical composition, comprising the RNA containing composition as defined herein and a pharmaceutically acceptable carrier and/or vehicle. Preferably the pharmaceutical composition is prepared for intratumoral application, preferably by injection into tumor tissue. Sterile injectable forms of the inventive pharmaceutical composition 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.

A pharmaceutically acceptable carrier typically includes the liquid or non-liquid basis of a composition comprising the components of the inventive composition. If the 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. 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, which are suitable for administration to a patient to be treated, may be used as well for the pharmaceutical composition according to the invention. The term "compatible" as used here means that these constituents of the inventive pharmaceutical composition are capable of being mixed with the components of the inventive pharmaceutical composition in such a manner that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the pharmaceutical composition under typical use conditions.

Administration:

The inventive composition or the inventive pharmaceutical composition may be administered by conventional needle injection or needle-free jet injection into the tumor tissue. In a preferred embodiment the inventive composition or the inventive pharmaceutical composition is administered by jet injection. Jet injection refers to a needle-free injection method, wherein a fluid comprising the inventive composition and, optionally, further suitable excipients is forced through an orifice, thus generating an ultra-fine liquid stream of high pressure that is capable of penetrating mammalian skin. In principle, the liquid stream forms a hole in the skin, through which the liquid stream is pushed into the target tissue, namely the tumor tissue. According to the invention, jet injection may be used for intratumoral application of the inventive composition.

The inventive composition may be administered by conventional needle injection or needle-free jet injection adjacent to and/or in close proximity to the tumor tissue. In a preferred embodiment the inventive pharmaceutical composition is administered by jet injection adjacent to and/or in close proximity to the tumor tissue. Jet injection refers to a needle-free injection method, wherein a fluid comprising the inventive composition and, optionally, further suitable excipients is forced through an orifice, thus generating an ultra-fine liquid stream of high pressure that is capable of penetrating mammalian skin. In principle, the liquid stream forms a hole in the skin, through which the liquid

stream is pushed into the target tissue, namely the tumor tissue. According to the invention, jet injection may be used for intratumoral application (adjacent to and/or in close proximity to the tumor tissue), particularily for injection of the inventive composition.

In other embodiments, the inventive composition or 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, intraarticular, intranodal, intrasynovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or infusion techniques.

Further particularly preferred administration routes are intradermal and intramuscular injection.

Despite, the inventive pharmaceutical composition may comprise further components for facilitating administration and uptake of components of the pharmaceutical composition. Such further components may be an appropriate carrier or vehicle, additional adjuvants for supporting any immune response, antibacterial and/or antiviral agents.

A further component of the inventive pharmaceutical composition may be an immunotherapeutic agent that can be selected from immunoglobulins, preferably IgGs, monoclonal or polyclonal antibodies, polyclonal serum or sera, etc. Preferably, such a further immunotherapeutic agent may be provided as a peptide/protein or may be encoded by a nucleic acid, preferably by a DNA or an RNA, more preferably an mRNA.

The inventive pharmaceutical composition typically comprises a “safe and effective amount” of the components of the inventive pharmaceutical composition, particularly of the RNA molecule(s) as defined herein. As used herein, a "safe and effective amount" means an amount of the RNA molecule(s) as defined herein as such that is sufficient to significantly induce a positive modification of the tumor or cancer disease. At the same time, however, a "safe and effective amount" is small enough to avoid serious side-effects and to permit a sensible relationship between advantage and risk. The determination of these limits typically lies within the scope of sensible medical judgment.

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.

Vaccine:

According to another particularly preferred aspect, the inventive compostion or the inventive pharmaceutical composition may be provided or used as a vaccine. Typically, such a vaccine is as defined above for pharmaceutical compositions. Additionally, such a vaccine typically contains the at least one RNA as defined herein or the inventive composition comprising a plurality of RNAs. Preferably, the at least one RNA encodes at least one tumor antigen or at least one immune activator as defined above. The inventive vaccine may also comprise a pharmaceutically acceptable carrier, adjuvant, and/or vehicle as defined herein 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 may be administered locally into tumor tissue.

The inventive vaccine can additionally contain one or more auxiliary substances in order to increase its immunogenicity or immunostimulatory capacity, if desired. Particularly preferred are adjuvants as auxiliary substances or additives as defined for the pharmaceutical composition.

Kit or kit of parts:

In a further aspect, the invention relates to a kit or kit of parts comprising the RNA containing composition as described above, or comprising the pharmaceutical composition as described above, or the components thereof and optionally technical instructions with information on the administration and dosage of the components.

Beside the components of the inventive RNA containing composition, the kit may additionally contain a pharmaceutically acceptable vehicle, an adjuvant and at least one further component e.g. an additional pharmaceutically active component/compound as defined herein, as well as means for administration and technical instructions. The components of the composition and possibly further components may be provided in lyophilized form. In a preferred embodiment, prior to use of the kit, the provided vehicle is than added to the lyophilized components in a predetermined amount as written e.g. in the provided technical instructions.

Medical indication:

The present invention furthermore provides several applications and uses of the inventive RNA containing composition, or the pharmaceutical composition, or the vaccine, or the kit or kit of parts as defined herein. As a main aspect of the invention the composition or the pharmaceutical composition or the kit or kit of parts may be used as a medicament, namely for treatment of tumor or cancer diseases. In this context the treatment is preferably done by intratumoral application, especially by injection into tumor tissue. According to another aspect, the present invention is directed to the second medical use of the RNA containing composition or the pharmaceutical composition, or the vaccine, or the kit or kit of parts as described above, wherein these subject matters are used for preparation of a medicament particularly for intratumoral application (administration) for treatment of tumor or cancer diseases.

Preferably, diseases as mentioned herein are selected from tumor or cancer diseases which preferably include e.g. Acute lymphoblastic leukemia, Acute myeloid leukemia, Adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, Anal cancer, Appendix cancer, Astrocytoma, Basal cell carcinoma, Bile duct cancer, Bladder cancer, Bone cancer, Osteosarcoma/Malignant fibrous histiocytoma, Brainstem glioma, Brain tumor, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, Breast cancer, Bronchial adenomas/carcinoids, Burkitt lymphoma, childhood Carcinoid tumor, gastrointestinal Carcinoid tumor, Carcinoma of unknown primary, primary Central nervous system lymphoma, childhood Cerebellar astrocytoma, childhood Cerebral astrocytoma/Malignant glioma, Cervical cancer, Childhood cancers, Chronic lymphocytic leukemia, Chronic myelogenous leukemia, Chronic myeloproliferative disorders, Colon Cancer, Cutaneous T-cell lymphoma, Desmoplastic small round cell tumor, Endometrial cancer, Ependymoma, Esophageal cancer, Ewing's sarcoma in the Ewing family of tumors, Childhood Extracranial germ cell tumor, Extragonadal Germ cell tumor, Extrahepatic bile duct cancer, Intraocular melanoma, Retinoblastoma, Gallbladder cancer, Gastric (Stomach) cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal stromal tumor (GIST), extracranial, extragonadal, or ovarian Germ cell tumor, Gestational trophoblastic tumor, Glioma of the brain stem, Childhood Cerebral Astrocytoma, Childhood Visual Pathway and Hypothalamic Glioma, Gastric carcinoid, Hairy cell leukemia, Head and neck cancer, Heart cancer, Hepatocellular (liver) cancer, Hodgkin lymphoma, Hypopharyngeal cancer, childhood Hypothalamic and visual

pathway glioma, Intraocular Melanoma, Islet Cell Carcinoma (Endocrine Pancreas), Kaposi sarcoma, Kidney cancer (renal cell cancer), Laryngeal Cancer, Leukemias, acute lymphoblastic Leukemia, acute myeloid Leukemia, chronic lymphocytic Leukemia, chronic myelogenous Leukemia, hairy cell Leukemia, Lip and Oral Cavity Cancer, Liposarcoma, Liver Cancer, Non-Small Cell Lung Cancer, Small Cell Lung Cancer, Lymphomas, AIDS-related Lymphoma, Burkitt Lymphoma, cutaneous T-Cell Lymphoma, Hodgkin Lymphoma, Non-Hodgkin Lymphomas, Primary Central Nervous System Lymphoma, Waldenström Macroglobulinemia, Malignant Fibrous Histiocytoma of Bone/Osteosarcoma, Childhood Medulloblastoma, Melanoma, Intraocular (Eye) Melanoma, Merkel Cell Carcinoma, Adult Malignant Mesothelioma, Childhood Mesothelioma, Metastatic Squamous Neck Cancer with Occult Primary, Mouth Cancer, Childhood Multiple Endocrine Neoplasia Syndrome, Multiple Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Diseases, Chronic Myelogenous Leukemia, Adult Acute Myeloid Leukemia, Childhood Acute Myeloid Leukemia, Multiple Myeloma (Cancer of the Bone-Marrow), Chronic Myeloproliferative Disorders, Nasal cavity and paranasal sinus cancer, Nasopharyngeal carcinoma, Neuroblastoma, Oral Cancer, Oropharyngeal cancer, Osteosarcoma/malignant fibrous histiocytoma of bone, Ovarian cancer, Ovarian epithelial cancer (Surface epithelial-stromal tumor), Ovarian germ cell tumor, Ovarian low malignant potential tumor, Pancreatic cancer, islet cell Pancreatic cancer, Paranasal sinus and nasal cavity cancer, Parathyroid cancer, Penile cancer, Pharyngeal cancer, Pheochromocytoma, Pineal astrocytoma, Pineal germinoma, childhood Pineoblastoma and supratentorial primitive neuroectodermal tumors, Pituitary adenoma, Plasma cell neoplasia/Multiple myeloma, Pleuropulmonary blastoma, Primary central nervous system lymphoma, Prostate cancer, Rectal cancer, Renal cell carcinoma (kidney cancer), Cancer of the Renal pelvis and ureter, Retinoblastoma, childhood Rhabdomyosarcoma, Salivary gland cancer, Sarcoma of the Ewing family of tumors, Kaposi Sarcoma, soft tissue Sarcoma, uterine Sarcoma, Sézary syndrome, Skin cancer (nonmelanoma), Skin cancer (melanoma), Merkel cell Skin carcinoma, Small intestine cancer, Squamous cell carcinoma, metastatic Squamous neck cancer with occult primary, childhood Supratentorial primitive neuroectodermal tumor, Testicular cancer, Throat cancer, childhood Thymoma, Thymoma and Thymic carcinoma, Thyroid cancer, childhood Thyroid cancer, Transitional cell cancer of the renal pelvis and ureter, gestational Trophoblastic tumor, Urethral cancer, endometrial Uterine cancer, Uterine sarcoma, Vaginal cancer, childhood Visual pathway and hypothalamic glioma, Vulvar cancer, Waldenström macroglobulinemia, and childhood Wilms tumor (kidney cancer).

Especially preferred examples of tumors or cancers that are suitable for intratumoral administration are prostate cancer, lung cancer, breast cancer, brain cancer, head and neck cancer, thyroid cancer, colon cancer, stomach cancer, liver cancer, pancreas cancer, ovary cancer, skin cancer, urinary bladder, uterus and cervix.

According to a specific embodiment, the medicament may be administered to the patient as a single dose or as several doses. In certain embodiments, the medicament may be administered to a patient as a single dose followed by a second dose later and optionally even a third, fourth (or more) dose subsequent thereto etc.

Preferably, the inventive composition is provided in an amount of at least 40 µg RNA per dose. More specifically, the amount of the mRNA comprised in a single dose is typically at least 200 µg, preferably from 200 µg to 1.000 µg, more preferably from 300 µg to 850 µg, even more preferably from 300 µg to 700 µg.

Treatment with additional (pharmaceutical) compounds:

In a particularly preferred embodiment the subject receiving the inventive composition, or the pharmaceutical composition or vaccine may be a patient with cancer or tumor who receives or received standard treatments of cancer. Preferably, the patient has achieved partial response or stable disease after having received standard treatments.

The standard treatments of cancer include chemotherapy, radiation, chemoradiation and surgery dependent on the particular cancer or tumor type to be treated, wherein these treatments are applied individually or in combination.

In some embodiments the subject receiving the inventive composition, pharmaceutical composition or vaccine may be a patient with cancer or tumor, who received or receives chemotherapy (e.g. first-line or second-line chemotherapy), radiotherapy, chemoradiation (combination of chemotherapy and radiotherapy), kinase inhibitors, antibody therapy and/or checkpoint modulators (e.g. CTLA4 inhibitors, PD1 pathway inhibitors), or a patient, who has

achieved partial response or stable disease after having received one or more of the treatments specified above.

In other embodiments the subject receiving the inventive composition, pharmaceutical composition or vaccine may be a patient with cancer or tumor, who received or receives an additional pharmaceutically active component/compound as defined above. Preferably, the subject is a patient, who has achieved partial response or stable disease after having received one or more of the treatments specified above.

According to a further aspect the invention refers to a method of treatment of tumor or cancer diseases, wherein the RNA containing composition as described above, or the pharmaceutical composition as described above, or the vaccine as described above, or the kit or kit of parts as described above is preferably applied intratumorally, especially by injection into tumor tissue. With respect to further features of the method for treatment it is referred to the description above.

Preferred intratumoral applications:

In this context it is particularly preferred that the intratumoral application of the RNA containing composition or the pharmaceutical composition or the vaccine as defined above is combined with the application of different agents/pharmaceutically active components/compounds. Particularly preferred are antibodies (e.g. check point modulators as e.g. anti-CTLA4, anti-OX40, anti-PD1 or anti-PD-L1) or ligands (e.g. CD40L).

In preferred embodiments the following combinations are particularly preferred:

- RNAdjuvant (i.t.) + anti-CTLA4 as protein (i.p./i.v.)
- RNAdjuvant (i.t.) + anti-CTLA4 as protein (i.t.)
- RNAdjuvant (i.t.) + anti-PD1 as protein (i.p./i.v.)
- RNAdjuvant (i.t.) + anti-PD1 as protein (i.t.)
- RNAdjuvant (i.t.) + anti-PD-L1 as protein (i.p./i.v.)
- RNAdjuvant (i.t.) + anti-PD-L1 as protein (i.t.)
- RNAdjuvant (i.t.) + CD40L (i.t.) as protein or encoded by a nucleic acid preferably an RNA, more preferably an mRNA

- RNAdjuvant (i.t.) + mRNA encoding IL-12 + mRNA encoding soluble PD-1 receptor + anti-CD73 (i.p./i.v.)
- RNAdjuvant (i.t.) + mRNA encoding IL-12 + mRNA encoding soluble PD-1 receptor + anti-CD137 (i.p./i.v.)
- RNadjuvant (i.t.)

(i.t. = intratumoral, i.p. = intraperitoneal, i.v. = intravenous)

In the present invention, if not otherwise indicated, different features of alternatives and embodiments may be combined with each other, where suitable. Furthermore, the term "comprising" shall not be narrowly construed as being limited to "consisting of" only, if not specifically mentioned. Rather, in the context of the present invention, "consisting of" is an embodiment specifically contemplated by the inventors to fall under the scope of "comprising", wherever "comprising" is used herein.

All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The examples and figures shown in the following are merely illustrative and shall describe the present invention in a further way. These figures and examples shall not be construed to limit the present invention thereto.

Short description of the figures:

Figure 1: shows survival proportions of mice bearing E.G7-OVA tumors after intratumoral treatment with mRNA encoding IL-12 (IL-12 mRNA) or with recombinant IL-12 protein (rIL-12 protein). The experiment was performed as described in Example 1. Kaplan-Meier survival curves are presented.

Figure 2: shows that intratumoral treatment of mice with a combination of IL-12 mRNA (R2763, SEQ ID NO: 1) and the polymeric carrier cargo complex (R2391, RNAdjuvant®, prepared as described in methods) led to a significantly decreased tumor volume compared to control groups. The experiment was performed as described in Example 2. Figure 2 shows the mean tumor volume at day 21 after tumor challenge (the last day when all animals were alive). Statistical analysis was performed in GraphPad Prism version 5.04 using Mann Whitney test.

Figure 3: shows survival proportions of mice bearing CT26 tumors after intratumoral treatment with a combination of IL-12 mRNA and the polymeric carrier cargo complex ("RNAdjuvant") as described in Example 2 and in legend of figure 2. Kaplan-Meier survival curves are presented. Statistical analysis was performed in GraphPad Prism version 5.04 using Log-rank test.

Figure 4: shows survival proportions of mice bearing CT26 tumors after intratumoral treatment with mRNA encoding the influenza nucleoprotein. The experiment was performed as described in Example 3. Kaplan-Meier survival curves are presented.

Figure 5: Panel (A) shows an analysis of the median tumor growth of mice bearing CT26 tumors after intratumoral treatment with mRNA encoding IL-12, RNAdjuvant, and mRNA encoding soluble PD-1. Respective combinations of these compounds, including control groups, were tested as indicated in the figure. The experiment was performed as described in Example 4.

Panel (B) shows survival proportions of mice bearing CT26 tumors after intratumoral treatment with mRNA encoding IL-12, RNAdjuvant, and mRNA encoding soluble PD-1. Respective combinations of these compounds, including control groups, were tested as indicated in the figure. The experiment was performed as described in Example 4. Kaplan-Meier survival curves are presented.

Figure 6: shows survival proportions of mice bearing CT26 tumors after intratumoral treatment with mRNA encoding IL-12, RNAdjuvant, mRNA encoding soluble PD-1

and intraperitoneal treatment of an anti-CD73 antibody. Respective combinations of these compounds, including control groups, were tested as indicated in the figure. The experiment was performed as described in Example 5. Kaplan-Meier survival curves are presented.

Figure 7: shows survival proportions of mice bearing CT26 tumors after intratumoral treatment with mRNA encoding IL-12, RNAdjuvant, mRNA encoding soluble PD-1 and intraperitoneal treatment of an anti-CD173 antibody. Respective combinations of these compounds, including control groups, were tested as indicated in the figure. The experiment was performed as described in Example 6. Kaplan-Meier survival curves are presented.

Figure 8: shows survival proportions of mice bearing CT26 tumors after intratumoral treatment with RNAdjuvant and intraperitoneal treatment of an anti-PD-1 antibody. Respective combinations of these compounds, including control groups, were tested as indicated in the figure. The experiment was performed as described in Example 7. Kaplan-Meier survival curves are presented.

Figure 9: shows survival proportions of mice bearing CT26 tumors after intratumoral treatment with mRNA encoding IL-12, RNAdjuvant, mRNA encoding CD40L compared to intratumoral treatment with mRNA encoding IL-12 alone. Respective combinations of these compounds, including control groups, were tested as indicated in the figure. The experiment was performed as described in Example 8. Kaplan-Meier survival curves are presented.

Figure 10: shows the mRNA sequence R3571 encoding murine CD40L (MmCD40L) according to SEQ ID NO. 10.073

Examples:

Methods: Preparation of the RNA

1. Preparation of DNA and RNA constructs

For the present examples DNA sequences encoding the indicated RNAs (see Table 17) were prepared and used for subsequent RNA *in vitro* transcription reactions.

Table 17: RNA constructs

RNA	Description	5'-UTR	3'-UTR	SEQ ID NO.
R1328	Murine IL-12 encoding mRNA (MmIL-12(GC))-sc-Flag)	-	Muag (3'-UTR of alpha globin)-A64-C30	SEQ ID NO: 1
R491	mRNA encoding Photinus pyralis luciferase (pPLuc (GC)) (irrelevant mRNA)	-	Muag (3'-UTR of alpha globin)-A64-C30	SEQ ID NO: 2
R2763	Murine IL-12 encoding mRNA (MmIL-12 (GC))	5'-TOP-UTR derived from the ribosomal protein 32L	albumin-3'-UTR-A64-C30-histone stem-loop	SEQ ID NO: 3
R2244	Luciferase encoding mRNA (PpLuc(GC))	5'-TOP-UTR derived from the ribosomal protein 32L	albumin-3'-UTR-A64-C30-histone stem-loop	SEQ ID NO: 4
R2025 R2391	Non-coding immunostimulatory RNA (RNAdjuvant) (SEQ ID NO. 118 of WO2009095226)			SEQ ID NO: 5
R2650 R2651	mRNA coding for the influenza nucleoprotein (H1N1(PR8)-NP(GC))	5'-TOP-UTR derived from the ribosomal protein 32L	albumin-3'-UTR-A64-C30-histone stem-loop	SEQ ID NO: 6
R3971	mRNA encoding solPD-1	5'-TOP-UTR derived from the ribosomal protein 32L	albumin-3'-UTR-A64-C30-histone stem-loop	SEQ ID NO: 389
R3571	mRNA encoding murine CD40L (MmCD40L)	5'-TOP-UTR derived from the ribosomal protein 32L	albumin-3'-UTR-A64-C30-histone stem-loop	SEQ ID NO: 10.073

The constructs of MmIL-12(GC), Influenza NP (GC), solPD-1 and PpLuc(GC)) were prepared by introducing a 5'-TOP-UTR derived from the ribosomal protein 32L, modifying the wild type coding sequence by introducing a GC-optimized sequence for stabilization, followed by a stabilizing sequence derived from the albumin-3'-UTR, a stretch of 64 adenosines (poly(A)-sequence), a stretch of 30 cytosines (poly(C)-sequence), and a histone stem loop. Most DNA sequences were prepared by modifying the wild type encoding DNA sequences by introducing a GC-optimized sequence for stabilization, using an *in silico* algorithm that increase the GC content of the respective coding sequence compared to the wild type coding sequence (in Table 12 indicated as "GC").

For the present example a DNA sequence encoding the non-coding immunostimulatory RNA (isRNA) R2025 was prepared and used for subsequent RNA *in vitro* transcription reactions.

2. RNA *In vitro* transcription

The respective DNA plasmids prepared according to section 1 above were transcribed *in vitro* using T7 polymerase. The RNA *in vitro* transcription reactions of the IL-12, the NP, PpLuc, CD40L and soluble PD-1 encoding constructs were performed in the presence of a CAP analog (m^7GpppG). The isRNA R2025 was prepared without CAP analog. Subsequently, the RNA was purified using PureMessenger® (CureVac, Tübingen, Germany; WO2008/077592A1).

3. Preparation of the polymeric cargo complex ("RNAdjuvant")

The following cationic peptide as cationic component of the polymeric carrier was used (Cys-Arg_{12}-Cys or $CR_{12}C$) according to SEQ ID NO: 7.

For synthesis of the polymeric carrier cargo complexes an RNA molecule having the RNA sequence R2025 as defined in section 1 above was mixed with the cationic $CR_{12}C$ peptide component as defined above. The specified amount of the RNA was mixed with the respective cationic component in mass ratios as indicated below, thereby forming a complex. If polymerizing cationic components were used according to the present invention, polymerization of the cationic components took place simultaneously to complexation of the nucleic acid cargo. Afterwards, the

resulting solution was adjusted with water to a final volume of 50 µl and incubated for 30 minutes at room temperature. Further details are described in WO2012013326.

The mass ratio of peptide:RNA was 1:3.7. The polymeric carrier cargo complex is formed by the disulfide-crosslinked cationic peptide $CR_{12}C$ as carrier and the immunostimulatory R2025 as nucleic acid cargo. This polymeric carrier cargo complex R2025/$CR_{12}C$ (designated R2391) was used as adjuvant in the following examples (referred to as "RNAdjuvant")

4. Preparation of the vaccine formulation coding for the influenza nucleoprotein (H1N1(PR8)-NP(GC)) (R2651)

The mRNA (R2650) was complexed with protamine by addition of protamine to the mRNA in the ratio (1:2) (w/w) (adjuvant component). After incubation for 10 min, the same amount of free mRNA used as antigen-providing mRNA was added. This vaccine formulation is termed herein R2651 (according to WO2010037539). The vaccine was administered in Ringer's Lactate solution.

5. Preparation of the RNA for administration

The naked (that is, non-formulated) PpLuc mRNA (R2244, R491)), IL-12 mRNA (R2763, R1328), soluble PD-1 mRNA (R3971), CD40L mRNA (R3571) were administered in Ringer's Lactate (RiLa) solution. The co-formulation of naked mRNAs and the polymeric carrier cargo complex "RNAdjuvant" (R2391) were also administered in Ringer's Lactate (RiLa) after mixing of both components directly before injection.

Example 1: Intratumoral application of mRNA coding for IL-12

5 female C57BL/6 mice per treatment group were inoculated with 10^6 cells E.G7-OVA cells 5 days before the first treatment. For each treatment group 5 established (about 100 mm^3) subcutaneously implanted EG.7-OVA tumors were treated. Tumors were treated with 16 µg mRNA coding for MmIL-12 (MmIL-12(GC))-sc-Flag) (R1328) or 0.5 µg MmIL-12 protein on d 0, 2, 4, 21, 23 and 25 with 50 µg (1 µg/µl). As control mice were treated with an irrelevant mRNA (pPLuc) (R491).

Study day 0 is defined as the first day of treatment. Tumor growth was monitored frequently (every 2-3 days). Mice with a volume of >3 cm^3 were killed.

Results of example 1

Figure 1 shows that the intratumoral treatment with the mRNA-encoded IL-12 (IL-12 mRNA) resulted in a significant increase in survival compared to the control group. Furthermore an increased survival could be observed compared to the intratumoral application of recombinant IL-12 protein (rIL-12 protein).

Example 2: Intratumoral treatment with mRNA encoding IL-12 in combination with an immunostimulating RNA (RNAdjuvant®)

The following table 18 summarizes the RNA constructs used for the example 2.

Table 18: RNA constructs for example 2

RNA	Description	Figure	SEQ ID NO.
R2763	Murine IL-12 encoding mRNA	1	SEQ ID NO. 1
R2244	Luciferase encoding mRNA (PpLuc)	2	SEQ ID NO. 2
R2025	Non-coding immunostimulatory RNA	3	SEQ ID NO. 3

Balb/c mice (n = 6 or 7, see table 14) were injected subcutaneously (s.c.) with $1x10^6$ CT26 cells (colon carcinoma cell line) per mouse (in a volume of 100 µl PBS) on the right flank on day 0 of the experiment. At day 9 after tumor challenge, mice were sorted according to the tumor size to obtain groups with a mean tumor volume of approximately 60 mm^3. Intratumoral (i.t.) therapy started at day 9 and continued for additional 4 injections every 3-4 days. Mice were injected with a combination of mRNA-encoded IL-12 (25 µg of R2763) + RNAdjuvant® (25 µg of R2391) (group A). To control for local inflammation due to RNA application or the injection procedure, mice were injected with control mRNA coding for luciferase (PpLuc, R2244, group B) or buffer (RiLa, group C), respectively. Untreated mice served as additional control (group D).

Tumor growth was monitored by measuring the tumor size in three dimensions using a calliper. Tumor volume was calculated according to the following formula:

$$volume\,(mm^3) = \frac{length\,(mm) \times \pi \times width^2\,(mm^2)}{6}$$

On day 9, 11, 14, 17 and 21 of the experiment mice were injected intratumorally (i.t.) with RNA according to the table 19 below. The volume for intratumoral injection was 50 µl.

Table 19: Animal groups

Group	Strain sex	Number of mice	RNA	Dose per mouse	Route, volume
A	BALB/c Female	7	R2763, R2391	25 µg of each RNA	i.t., 50 µl
B	BALB/c Female	7	R2244	50 µg	i.t., 50 µl
C	BALB/c Female	6	RiLa	---	i.t., 50 µl
D	BALB/c Female	6	---	---	---

Results of example 2

Figure 2 shows that the intratumoral treatment with the combination of mRNA-encoded IL-12 (R2763) and RNAdjuvant® (R2391) resulted in a statistically significant decrease in tumor volume at day 21 after tumor challenge compared to all control groups.

Figure 3 shows that the intratumoral treatment with the combination of mRNA-encoded IL-12 (R2763) and RNAdjuvant® (R2391) resulted in a statistically significant increase in survival compared to all three control groups (group A vs. group B * p=0.0104, group A vs. group C ** p=0.0035, group A vs. Group D * p==0.0263).

Example 3: Vaccination of mice with mRNA encoding the influenza nucleoprotein (NP) and subsequent intratumoral treatment with NP-encoding mRNA

The objective of this experiment was to test whether a pre-existing immune response can be harnessed against an established tumor. To this end, mice were first vaccinated with RNActive

(vaccine formulation complexed with protamine) encoding the influenza nucleoprotein (NP) (R2651) which induces a high level of anti-NP $CD8^+$ T cell responses, then challenged with CT26 tumor cells followed by intratumoral treatment with naked RNA encoding NP (R2650).

27 Balb/c mice were vaccinated intradermally (i.d.) with 40 µg of H1N1(PR8)-NP(GC) RNActive (R2651) (2 x 50 µl) or Ringer-Lactate buffer (RiLa) as control on day 0, day 7 and day 16 of the experiment. On day 14 all mice were challenged subcutaneously (s.c.) with 1 x 10^6 CT26 cells per mouse (in a volume of 100 µl PBS) on the right flank. On day 22, mice were assigned to the different groups as shown in Table 20.

On day 23, seven days after the second boost, intratumoral (i.t.) application of 50 µg naked H1N1(PR8)-NP(GC) mRNA (R2650) started (only group C) and continued for additional four injections (at day 25, day 28, day 31 and day 35). The volume for intratumoral injection was 50 µl. A detailed treatment schedule is shown in Table 21.

Tumor growth was monitored by measuring the tumor size in three dimensions using a calliper. Tumor volume was calculated according to the following formula:

$$volume\,(mm^3) = \frac{length\,(mm) \times \pi \times width^2\,(mm^2)}{6}$$

Table 20: Animal groups

Group	Strain sex	Number of mice	mRNA i.d.	mRNA i.t.
A	BALB/c Female	9	RiLa	---
B	BALB/c Female	9	R2651 (40 µg)	---
C	BALB/c Female	9	R2651 (40 µg)	R2650 (50 µg)

Table 21: Vaccination schedule

Day	Treatment
0	i.d. vaccination all groups
7	i.d. vaccination all groups
14	Tumor challenge of all groups (1×10^6 CT26 cells/mouse)
16	i.d. vaccination all groups
23	i.t. vaccination group C
25, 28, 31, 35	i.t. vaccination group C

Results of Example 3

Figure 4 shows that pre-existent immunity (induced in this model by the NP vaccination) increased the median survival time (MST) of mice which received intratumoral application of NP-encoded mRNA compared to mice which were treated with buffer only (MST=28 vs. MST=21, respectively).

Example 4: Intratumoral treatment with an immunostimulating RNA ("RNAdjuvant") and an mRNA encoding soluble PD-1 and and an mRNA encoding IL-12

Table 22 summarizes the treatment as used in the present example. RNAdjuvant and the mRNA constructs encoding IL-12 and soluble PD-1 were administered intratumorally (i.t.). In CT26 tumor challenged mice, survival rates and median tumor growth were analyzed.

Table 22: Groups, treatment and RNA dilution

Group	Nr. of mice	i.t. treatment (25µg for each component)	Vaccination schedule
A	10	IL-12 + RNAdjuvant + soluble PD-1	2X week
B	10	IL-12	2X week
C	10	RNAdjuvant	2X week
D	10	RiLa	2X week

Tumor challenge and administration of the inventive composition:

60 Balb/c mice were challenged subcutaneously with $1x10^6$ CT26 cells per mouse (volume in 100µl PBS) on the right flank on day 0 of the experiment. On day 8 mice were sorted according to tumor size. According to tumor size, the first vaccination took place on day 8 or 9 (tumors should have a size of about 40-50mm^3). Mice were vaccinated with different combinations of mRNAs and RNAdjuvant according to the table above. Six vaccinations took place. Volume for intratumoral injection was 50µl.

Mice were injected according to the indicated scheme shown in Table 22. Median tumor growth was determined according to example 3. The results of the experiment are shown in Figure 5, wherein Figure 5A shows the effect of the inventive composition on tumor growth, and Figure 5B shows the effect of the inventive composition on survival.

Results:

The results in Figure 5A show that the inventive composition comprising an mRNA encoding IL-12 and mRNA encoding soluble PD-1 in combination with RNAdjuvant (group "A" according to Table 22) strongly decreased the median tumor volume compared to the other treatments (groups B-D according to Table 22). In addition, the results in Figure 5B show that the inventive composition comprising an mRNA encoding IL-12 and mRNA encoding soluble PD-1 in combination with RNAdjuvant (group "A" according to Table 22) strongly increased the survival of tumor challenged mice compared to the other treatments (groups B-D according to Table 22).

Example 5: Intratumoral treatment with mRNA encoding IL-12 in combination with an immunostimulating RNA ("RNAdjuvant") and mRNA encoding sol PD-1 and anti-CD73 antibody

Table 23 summarizes the treatment as used in the present example. In addition to RNAdjuvant and mRNA constructs encoding IL-12 and soluble PD-1 (administered intratumorally (i.t.)), an anti CD73 antibody (BioXCell) was co-administered intraperitoreally (i.p.). In CT26 tumor challenged mice, survival rates were analyzed.

Table 23: Groups, treatment and RNA dilution

Group	Nr. of mice	i.t. treatment (25µg for each component)	i.p. treatment	Vaccination schedule
A	10	IL-12 + RNAdjuvant + soluble PD-1	a-CD73	2X week
B	10	IL-12 + RNAdjuvant+ soluble PD-1	Rat IgG2a	2X week
C	10	RiLa	a-CD73	2X week
D	10	RiLa	Rat IgG2a	2X week

Tumor challenge and administration of the inventive composition:

The tumor challenge was performed according to the previous experiments (see Example 4). Mice were injected according to the indicated scheme shown in Table 23.

The results of the experiment are shown in Figure 6.

Results:

Figure 6 shows that the intratumoral treatment with mRNA-encoded IL-12 (R2763), mRNA encoded sol-PD-1 (R3971) and RNAdjuvant® (R2391) in combination with an i.p. administration of anti CD73 antibody (Group "A" according to Table 23) resulted in a statistically significant increase in survival compared to the relevant control group that only received an anti CD73 antibody (Group "C" according to Table 23) and in an increase in survival rates compared to the the treatment with IL-12 + RNAdjuvant + soluble PD-1 and a control antibody (Rat IgG2a, BioXCell) (Group "B" according to Table 23).

Example 6: Intratumoral treatment with mRNA encoding IL-12 in combination with an immunostimulating RNA ("RNAdjuvant") and an anti-CD137 antibody

Table 24 summarizes the treatment as used in the present example. In addition to RNAdjuvant and the mRNA constructs encoding IL-12 and soluble PD-1 (administered intratumorally (i.t.)), an anti CD137 antibody (BioXCell) was co-administered intraperitoreally (i.p.). In CT26 tumor challenged mice, survival rates were analyzed.

Table 24: Groups, treatment and RNA dilution

Group	Nr. of mice	i.t. treatment (25µg)	i.p. treatment	Vaccination schedule
A	10	IL-12 + RNAdjuvant + soluble PD-1	a-CD137	2X week
B	10	IL-12 + RNAdjuvant+ soluble PD-1	Rat IgG2a	2X week
C	10	RiLa	a-CD137	2X week
D	10	RiLa	Rat IgG2a	2X week

Tumor challenge and administration of the inventive composition:

The tumor challenge was performed according to the previous experiments (see Example 4).

Mice were injected according to the indicated scheme shown in Table 24.

The results of the experiment are shown in Figure 7.

Results:

Figure 7 shows that the intratumoral treatment with mRNA-encoded IL-12 (R2763) sol-PD-1 (R3971) and RNAdjuvant® (R2391) in combination with an i.p. administration of anti CD-137 antibody (Group "A" according to Table 24) resulted in a significant increase in survival compared to the relevant control group that only received the antibody anti CD-137 (Group "C" according to Table 24) and in an increase in survival rates compared to the the treatment with IL-12 + RNAdjuvant + soluble PD-1 and a control antibody (Rat IgG2a, BioXCell) (Group "B" according to Table 24).

Example 7: Treatment with with an immunostimulating RNA ("RNAdjuvant") in combination with a checkpoint inhibitor anti PD-1 antibody

Table 25 summarizes the treatment as used in the present example. In addition to RNAdjuvant (administered i.t.), a checkpoint inhibitor anti PD-1 (BioXCell) was administered i.p. In CT26 tumor challenged mice, survival rates were analyzed.

Table 25: Groups, treatment and RNA dilution /antibody dilution

Group	Construct	Antibody	Amount of RNA (µg)	Vaccination schedule
A	RiLa (i.t.)	---		2X week
B	RNAdjuvant (i.t.)	Control Ab (i.p.)(100µg)	25	2X week
C	RNAdjuvant (i.t.)	Anti-PD-1 (i.p.) (200µg)	25	2X week
D	RiLa (i.t.)	Anti-PD-1 (i.p.) (200µg)	---	2X week

Tumor challenge and administration of the inventive composition:

The tumor challenge was performed according to the previous experiments (see Example 4). Mice were injected according to the indicated scheme shown in Table 25.

The results of the experiment are shown in Figure 8.

Results:

Figure 8 shows that the intratumoral (i.t.) treatment with RNAdjuvant® (R2391) in combination with an i.p. administration of anti PD-1 antibody (Group "C" according to Table 25) resulted in an increase in survival compared to the relevant control group that only received the checkpoint inhibitor anti PD-1 antibody (Group "D" according to Table 25) and in an increase in survival rates compared to the treatment with RNAdjuvant and a control antibody (anti hamster IgG, BioXCell) (Group "B" according to Table 25).

Example 8: Intratumoral treatment with an immunostimulating RNA ("RNAdjuvant") and an mRNA encoding CD40 ligand (CD40L) and an mRNA encoding IL-12

Table 26 summarizes the treatment as used in the present example. RNAdjuvant and the mRNA constructs encoding IL-12 and murine CD40L were administered intratumorally (i.t.). In CT26 tumor challenged mice, survival rates were analyzed.

Table 26: Groups, treatment and RNA dilution

Group	Nr. of mice	i.t. treatment (25µg per RNA)	Vaccination schedule
A	8	IL-12 + RNAdjuvant + CD40L	2X week
B	8	IL-12	2X week
C	8	RiLa	2X week

Tumor challenge and administration of the inventive composition:

The tumor challenge was performed according to the previous experiments (see Example 4).

Mice were injected according to the indicated scheme shown in Table 26.

The results of the experiment are shown in Figure 9.

Results:

The results in Figure 9 show that the inventive composition comprising an mRNA encoding IL-12 and an mRNA encoding CD40L in combination with RNAdjuvant (group "A" according to table 26) strongly increased the median survival of tumor challenged mice compared to the other treatments (groups B-C according to table 26).

Claims

1. RNA containing composition comprising at least one non-coding, immunostimulating RNA for use in the treatment or prophylaxis of tumor and/or cancer diseases,
wherein the RNA containing composition is to be applied intratumorally especially by injection into tumor tissue, and
wherein the treatment or prophylaxis comprises a PD-1 inhibitor or a PD-L 1 inhibitor to be administered, wherein the PD-1 inhibitor is an antagonistic antibody directed against PD-1 and the PD-L1 inhibitor is an antagonistic antibody directed against PD-L1.

2. Use of an RNA containing composition comprising at least one non-coding, immunostimulating RNA in the manufacture of a medicament for the treatment or prophylaxis of tumor and/or cancer diseases,
wherein the RNA containing composition is to be applied intratumorally especially by injection into tumor tissue, and
wherein the treatment or prophylaxis comprises a PD-1 inhibitor or a PD-L 1 inhibitor to be administered, wherein the PD-1 inhibitor is an antagonistic antibody directed against PD-1 and the PD-L1 inhibitor is an antagonistic antibody directed against PD-L1.

3. The RNA containing composition for use of claim 1, or the use of an RNA containing composition of claim 2, wherein the immunostimulating RNA comprises at least one RNA sequence according to formula (III) (GlXmGn), formula (IV) (ClXmCn), formula (V) (NuGlXmGnNv)a, and/or formula (VI) (NuClXmCnNv)a).

4. The RNA containing composition for use of claim 1 or 3, or the use of an RNA containing composition of claim 2 or 3, wherein the immunostimulating RNA comprises at least one RNA sequence according to SEQ ID NO.5, 394 and 10072.

5. The RNA containing composition for use of any of claims 1, 3 and 4, or the use of an RNA containing composition of any of claims 2 to 4, wherein the at least one RNA is complexed with one or more cationic or polycationic compounds, preferably with cationic or polycationic polymers, cationic or polycationic peptides or proteins, e.g. protamine, cationic or polycationic polysaccharides and/or cationic or polycationic lipids.

6. The RNA containing composition for use of claim 5, or the use of an RNA containing composition of claim 5, wherein the cationic or polycationic compound is a polymeric carrier.

7. The RNA containing composition for use of claim 6, or the use of an RNA containing composition of claim 6, wherein the polymeric carrier is formed by disulfide-crosslinked cationic components, preferably disulfide-crosslinked cationic peptides, preferably comprising peptides according to formula VII, VIIa and/or VIIb and/or a compound according to formula (VIII) (L-P1-S-[S-P2-S] n-S-P3-L).

8. The RNA containing composition for use of any of claims 5 to 7, or the use of an RNA containing composition of any of claims 5 to 7, wherein the N/P ratio of the at least one RNA to the one or more cationic or polycationic compounds, preferably cationic or polycationic peptides or proteins is in the range of about 0.1 to 10, including a range of about 0.3 to 4, of about 0.5 to 2, of about 0.7 to 2 and of about 0.7 to 1.5.

9. The RNA containing composition for use of any of claims 1, 3 to 8, or the use of an RNA containing composition of any of claims 2 to 8, wherein the RNA containing composition comprises at least one RNA, which is complexed with one or more cationic or polycationic compounds, and at least one free RNA, preferably coding RNA, more preferably mRNA.

10. The RNA containing composition for use of any of claims 1, 3 to 9, or the use of an RNA containing composition of any of claims 2 to 9, wherein the at least one mRNA is complexed with one or more lipids and thereby forming liposomes, lipid nanoparticles and/or lipoplexes.

11. The RNA containing composition for use of any of claims 1, 3 to 8, or the use of an RNA containing composition of any of claims 2 to 10, wherein the RNA containing composition comprises a polymeric carrier cargo complex, formed by a polymeric carrier, preferably comprising disulfide-crosslinked cationic peptides, preferably Cys-Arg_{12}, and/or Cys-Arg_{12}-Cys, and an immunostimulating RNA, preferably the RNA sequence according to SEQ ID NO: 5, 394 or 10072.

12. Pharmaceutical composition comprising the RNA containing composition as defined according to claims 1, 3 to 11, and a pharmaceutically acceptable carrier and/or vehicle for use in the treatment or prophylaxis of tumor and/or cancer diseases, wherein the pharmaceutical composition is to be applied intratumorally especially by injection into tumor tissue, wherein the treatment or prophylaxis comprises the administration of a PD-1 inhibitor or a PD-L1 inhibitor, wherein the PD-1 inhibitor is an antagonistic antibody directed against PD-1 and the PD-L1 inhibitor is an antagonistic antibody directed against PD-L1.

13. Use of an RNA containing composition of claims 2 to 11, and a pharmaceutically acceptable carrier and/or vehicle in the manufacture of a medicament for the treatment or prophylaxis of tumor and/or cancer diseases, wherein the pharmaceutical composition is to be applied intratumorally especially by injection into tumor tissue, wherein the treatment or prophylaxis comprises a PD-1 inhibitor or a PD-L1 inhibitor to be administered, wherein the PD-1 inhibitor is an antagonistic antibody directed against PD-1 and the PD-L1 inhibitor is an antagonistic antibody directed against PD-L1.

14. Kit or kit of parts comprising the RNA containing composition as defined according to claims 1, 3 to 11, or the pharmaceutical composition as defined according to claim 12, and optionally technical instructions with information on the dosage to be administered, for use in the treatment or prophylaxis of tumor and/or cancer diseases,
wherein the RNA containing composition or the pharmaceutical composition is to be applied intratumorally especially by injection into tumor tissue,

wherein the treatment or prophylaxis comprises a PD-1 inhibitor or a PD-L 1 inhibitor to be administered, wherein the PD-1 inhibitor is an antagonistic antibody directed against PD-1 and the PD-L1 inhibitor is an antagonistic antibody directed against PD-L 1.

15. The RNA containing composition for use of any of claims 1, 3 to 11, the pharmaceutical composition for use of claim 12, the use of an RNA containing composition of claims 2 to 13, or the kit or kit of parts for use of claim 14, wherein the treatment further comprises radiation therapy and/or surgery.