Expression Systems

Field of the Invention

The invention relates to an expression system comprising polynucleotides encoding proteins, wherein the expression system comprises a first polynucleotide encoding at least one protein, peptide or variant thereof, which induces a T cell response, and a second polynucleotide encoding at least one protein peptide or variant thereof, which induces an anti-pathogenic B cell response. The invention further relates to protein mixtures encoded by the expression system and cells comprising the expression system or the protein mixture and pharmaceutical compositions comprising the expression system or the protein mixture. The expression system, polynucleotides, proteins, cells, and pharmaceutical compositions are useful in the prophylaxis or treatment of infections. The invention further relates to nucleotide constructs and expression systems encoding a modified influenza hemagglutinin (HA).

Background of the Invention

Infectious diseases are still a major thread of mankind. One way for preventing or treating infectious diseases is the artificial induction of an immune response by vaccination which is the administration of antigenic material to an individual such that an adaptive immune response against the respective antigen is developed. The antigenic material may be pathogens (e.g. microorganisms or viruses) which are structurally intact but inactivated (i.e. non-infective) or which are attenuated (i.e. with reduced infectivity), or purified components of the pathogen that have been found to be highly immunogenic. Another approach for inducing an immune response against a pathogen is the provision of expression systems comprising one or more vector encoding immunogenic proteins or peptides of the pathogen. Such vector may be in the form of naked plasmid DNA, or the immunogenic proteins or peptides are delivered by using viral vectors, for example on the basis of modified vaccinia viruses (e.g. Modified Vaccinia Ankara; MVA) or adenoviral vectors. Such expression systems have the advantage of comprising well-characterized components having a low sensitivity against environmental conditions.

It is a particular aim when developing vector based expression systems that the application of these expression systems to a patient elicits an immune response which is protective against the infection by the respective pathogen. However, although inducing an immunogenic response against the pathogen, some expression systems are not able to elicit an immune response which is strong enough to fully protect against infections by the pathogen.

Accordingly, there is still a need for expressions systems which are capable of inducing a protective immune response against a pathogen, e.g. an infectious agent like a virus.

Viruses

Viruses are a group of pathogens/infectious agents which have no own metabolism and can be considered as obligatory endoparasites of the respective host cells using at least parts of host's cell facilities for conducting viral protein expression and virus replication. Viruses can be classified on the basis of the type (DNA/RNA), the strandedness (single-stranded (ss) or double-stranded (ds)), the sense (negative sense or positive sense) of the nucleic acid constituting their genome and their replication (Baltimore classification). Accordingly, viruses are generally classified in DNA and RNA viruses. Viruses can be further classified into single-stranded (ss) or double-stranded (ds) DNA or RNA viruses, which genome is a single-stranded or double-stranded nucleic acid. Some viruses have a genome which is partially double-stranded and partially single-stranded (e.g. hepadnaviruses). The orientation or "sense" of the genome and/or in the manufacturing of medicament for use in the prophylaxis or treatment of a pathogen and/or for use in methods of prophylaxis or treatment of of a pathogen, wherein the pathogen plays an important role for the viral life cycle of viruses, in particular in the life cycle of ssRNA viruses or ssDNA viruses. A positive sense ssRNA (+) genome has the same orientation as a cellular mRNA and can be directly translated into viral proteins. In the life cycle of viruses having a negative sense single-stranded RNA genome (ssRNA (-)), it is necessary that the genomic sequences are transcribed into positive sense mRNA which can be translated into viral proteins by the host cell. A single-stranded genome that contains both positive-sense and negative-sense is called "ambisense" (e.g. ssRNA (+/-), ssDNA(+/-)).

Although the genome of viruses may be quite large (e.g. in the case of DNA viruses), in particular small RNA viruses have evolutionary developed strategies for expressing their gene products (e.g. proteins and peptides) in a very efficient manner. One of these strategies is the expression of one or more polyprotein encoded by the viral genome, which is co- or posttranslationally processed into single proteins and/or peptides. This strategy is adapted, for example, by some double-stranded (ds) RNA viruses or single-stranded (ss) RNA viruses having a positive sense genome. "Enveloped viruses", such as orthomyxoviruses, paramyxoviruses, retroviruses, flaviviruses, rhabdoviruses and alphaviruses, are surrounded by a lipid bilayer originating from the host plasma membrane (1).

Attachment glycoproteins are found in all enveloped viruses and mediate the initial interaction between the viral envelope and the plasma membrane of the host cell via their binding to carbohydrate moieties or cell adhesion domains of proteins or other molecules on the plasma membrane of the host cell. Thereby, attachment glycoproteins bridge the gap between the

virus and the membrane of the host cell. Attachment glycoproteins designated as "H" possess hemagglutinin activity, glycoproteins designated as "HN possesses hemagglutinin and neuraminidase activities. Attachment glycoproteins are designated as "G" when they have neither haemagglutination nor neuraminidase activity.

Paramyxoviruses

Paramyxoviruses are a family of animal viruses which comprises a single stranded non-segmented negative-sense RNA. Paramyxoviruses are responsible for a number of animal and human diseases. The RNA genome of paramyxoviruses is 15-19 kilo bases (kb) in length and encodes 6-10 genes. Each gene contains transcription start/stop signals at the beginning and end, which are transcribed as part of the gene. The gene sequence is conserved across the paramyxoviruses due to a phenomenon known as transcriptional polarity in which genes closest to the 3' end of the genome are transcribed in greater abundance than those towards the 5' end. After each gene is transcribed, the RNA-Dependent RNA polymerase pauses to release the new mRNA when it encounters an intergenic sequence. When the RNA polymerase is paused, there is a chance that it will dissociate from the RNA genome. If it dissociates, it must reenter the genome at the leader sequence, rather than continuing to transcribe the length of the genome. As a result, the further downstream genes are from the leader sequence, the less they will be transcribed by the RNA polymerase. The genes of paramyxoviruses are arranged in relative order of protein needed for successful infection. The conserved gene sequence is Nucleocapsid – Phosphoprotein – Matrix – Fusion – Attachment – Large (polymerase).

Many Paramyxovirus genomes follow the so-called "Rule of Six". According to this rule, the total length of the genome is almost always a multiple of six. However, the members of the sub-family Pneumovirinae comprising the Respiratory Syncytial Virus (RSV) do not follow this rule.

Respiratory syncytial virus (RSV)

The enveloped virus designated as respiratory syncytial virus (RSV) is the most important cause of viral lower respiratory tract illness (LRTI) in infants and children worldwide (2). In the United States, it is estimated that 70,000–126,000 infants are hospitalized annually with RSV pneumonia or bronchiolitis and that the rate of hospitalization for bronchiolitis has increased since 1980 (3). Children are infected by 2 years of age and the WHO has estimated that RSV causes disease in approximately 64 million children each year and 160,000 deaths. In industrialised countries, RSV is responsible for at least 50% of hospitalisations for respiratory disease in children, and up to 6% of all RSV infections in children result in hospitalisation (4). RSV infection does not provoke lasting immunity, so that human hosts experience lifelong cycles of infection and re-infection. Although it is traditionally regarded as a pediatric pathogen,

RSV also causes severe disease in the elderly and immuno-compromised individuals (5). The burden of RSV disease in the elderly is comparable to that of seasonal influenza and the economic impact of RSV-related disease in adults is estimated to be greater than that of influenza in relation to numbers of days lost from work (6, 7). Monoclonal antibody prophylaxis is effective in reducing RSV hospitalisations by 50% in infants at high risk of severe disease (8). However, there is currently no effective RSV vaccine or anti-viral therapy.

The disastrous effect of a formalin-inactivated (FI) RSV vaccine in infants in the 1960s has hampered vaccine development. The vaccine failed to protect against RSV infection and induced exacerbated respiratory disease (9) which has been attributed to induction of high titre, poorly neutralising, low affinity antibodies, lack of CD8+ T cell priming and induction of a Th2-biased immune response (10, 11, and 12). There is evidence that RSV impairs the induction of an adequate adaptive T cell immune response (13).

There is, therefore, a clear need for an effective vaccine not only to protect infants, but also to boost immunity in the elderly and to reduce the circulation of RSV in siblings and adults, who are the main source of RSV infection for infants. A RSV vaccine capable of inducing neutralizing antibody response and potent and broad T cell response for priming a T cell responses in individuals who have not yet been infected with RSV (infants) or for boosting a pre-existing T cell response in individuals who need to 'reset' the memory response to higher levels (elderly) is especially desirable.

Orthomyxoviruses

Orthomyxoviruses are a family of RNA viruses that includes five genera: Influenzavirus A, Influenzavirus B, Influenzavirus C, Isavirus and Thogotovirus. A sixth genus has recently been described. The first three genera contain viruses that cause influenza in vertebrates, including birds, humans, and other mammals. The three genera of influenza virus have antigenic differences in their nucleoprotein and matrix protein. Influenzavirus A infects humans, other mammals, and birds, and causes all influenza pandemics. Influenzavirus B infects humans and seals. Influenzavirus C infects humans and pigs.

Viruses of the Orthomyxovirus family contain 6 to 8 segments of linear negative-sense single stranded RNA. The total genome length is 12000-15000 nucleotides (nt). The largest segment 2300-2500 nt; of second largest 2300-2500 nt; of third 2200-2300 nt; of fourth 1700-1800 nt; of fifth 1500-1600 nt; of sixth 1400-1500 nt; of seventh 1000-1100 nt; of eighth 800-900 nt. Genome sequence has terminal repeated sequences; repeated at both ends. Terminal repeats at the 5'-end are 12-13 nucleotides long. The nucleotide sequences of 3'-terminus are identical the same in genera of same family; most on RNA (segments), or on all RNA species. Terminal repeats at the 3'-end 9-11 nucleotides long. .

Influenza virus is one of the most important respiratory pathogens. In the US alone, influenza infection is responsible for 20,000-40,000 deaths and over 100,000 hospitalizations annually (1). Infants, the elderly, and individuals with compromised cardiac, pulmonary, or immune systems are at great risk of serious complications following flu infection.

Immunization proves to be the most effective measure in preventing the disease. One of the common features shared by all current influenza vaccines consists in targeting primarily the induction of neutralizing antibodies directed against the major viral envelope protein, hemagglutinin (HA).

Summary of the Invention

The invention provides in a first aspect an expression system comprising a first polynucleotide encoding at least one protein, peptide or variant thereof, which induces a T cell response and a second polynucleotide encoding at least one protein, peptide or variant thereof, which induces an anti-pathogenic B cell response.

In a second aspect, the invention provides an isolated protein mixture encoded by the expression system of the first aspect.

In a third aspect, the invention provides an isolated host cell containing the expression system of the first aspect and/or the protein mixture of the second aspect.

In a fourth aspect, the present invention provides a composition comprising the expression system of the first aspect, or the protein mixture of the second aspect, and a pharmaceutical acceptable carrier and/or excipient.

In a fifth aspect, the present invention provides the expression system of the first aspect, the protein mixture of the second aspect, the cell of the third aspect and the composition of the fourth aspect, for the use in medicine in particular in the treatment or prevention of infectious diseases, preferably a viral disease.

In a sixth aspect, the present invention provides for a method of treatment or prevention of a viral disease comprising the administration of an effective amount of the expression system of the first aspect, the protein mixture of the second aspect, the cell of the third aspect and the composition of the fourth aspect.

In a seventh aspect, the present invention provides for a method of enhancing an immune response comprising the administration of the expression system of the first aspect, the protein mixture of the second aspect, the cell of the third aspect and the composition of the fourth aspect.

In an eighth aspect, the present invention provides nucleotide constructs encoding influenza hemagglutinin (HA), an expression system comprising these nucleotide constructs, and proteins or polyproteins encoded by the nucleotide constructs or the expression system, wherein the HA0 cleavage site has a multibasic sequence.

In a ninth aspect, the present invention provides the use of the multibasic HA0 cleavage site for constructing expression systems capable for expressing influenza hemagglutinin (HA) *in vitro* and/or *in vivo*.

In a tenth aspect, the invention provides an isolated protein mixture encoded by the expression system of the eighth aspect.

In an eleventh aspect, the invention provides an isolated host cell containing the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect and/or the protein mixture of the tenth aspect.

In a twelfth aspect, the present invention provides a composition comprising the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect, or the protein mixture of the tenth aspect, and a pharmaceutical acceptable carrier and/or excipient.

In a thirteenth aspect, the present invention provides the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect, the protein mixture of the tenth aspect, the cell of the eleventh aspect and the composition of the twelfth aspect, for the use in medicine in particular in the treatment or prevention of influenza virus infections.

In a fourteenth aspect, the present invention provides for a method of treatment or prevention of an influenza virus infections comprising the administration of an effective amount of the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect, the protein mixture of the tenth aspect, the cell of the eleventh aspect and the composition of the twelfth aspect.

In a fifteenth aspect, the present invention provides for a method of enhancing an immune response comprising the administration of the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect, the protein mixture of the tenth aspect, the cell of the eleventh aspect and the composition of the twelfth aspect.

The above summary does not necessarily describe all aspects of the present invention.

Brief Description of the Figures

Fig. 1: Schematic Diagram of the RSV vaccine polyprotein. conF0 = consensus sequence of the F protein, 2A = translational cleavage site of the Foot and Mouth Disease virus, conN = consensus sequence of the N protein, conM2-1 = consensus of the M2-1 protein.

Fig. 2: The vaccine antigen F0ΔTM-N-M2-1 is efficiently processed in mammalian cells. Western Blot analysis of lysates from HeLa cells; nt: not transfected Hela. RSV transf: HeLa cells transfected with F0ΔTM-N-M2-1. RSV inf: Hep2 cells infected with RSV strain A

Fig. 3: The secreted F protein forms a homotrimer. Western Blot analysis of supernatant from transfected HeLa cells; RSV: F0ΔTM-N-M2-1 transfected, F0: F0ΔTM transfected, Ctrl: empty plasmid transfected

Fig. 4: The F protein expressed from the vaccine polyprotein is a better immunogen than the F protein alone. A. Western Blot analysis of supernatant from HeLa cells infected with PanAd3/ F0ΔTM-N-M2-1 which was probed with different dilutions of sera from mice immunized with F0ΔTM or F0ΔTM-N-M2-1 **B.** densitometric scanning of the Western Blot on panel A. Data are expressed as Relative Intensity of the area corresponding to the protein band.

Fig. 5: The RSV vaccine induced potent systemic T cell immunity in mice by a single intramuscular injection. IFNg-Elispot assay of splenocytes of PanAd3/ F0ΔTM-N-M2-1 immunized Balb/C mice using mapped immunodominant peptides from RSV F and M proteins.

Fig. 6: Schematic diagram of the Influenza vaccine polyprotein. NP = consensus sequence of the NP protein, M1= consensus sequence of M1 protein, 2A = translational cleavage site of the Foot and Mouth Disease virus, H1p = consensus sequence of the HA protein from H1N12009.

Fig. 7 Western Blot analysis of H1p expression in transfected HeLa cells. Total lysate of HeLa cells transfected with PVJ-H1p (Lane 1), with PVJ-H1 (Lane 2) and not transfected CTR (Lane 3). The arrows show the bands corresponding to the uncleaved (70kD) HA0 form and the cleaved (28 Kd) HA2. The polyclonal anti-HA serum recognize epitopes in the HA2 protein fragment. It is shown that theH1p protein is fully processed.

Fig. 8 Whole-cell FACS analysis of membrane-displayed HA proteins. The histograms represent the median fluorescence analysis of HeLa cells transfected with wild type HA (right upper panel) and H1 (right lower panel). Cells were incubated with hyperimmune mouse polyclonal serum raised against H1p and then with a secondary anti-mouse antibody PE-conjugated. In the left upper and lower panels, cells were incubated with mouse pre-immune serum to set the background fluorescence level.

Fig. 9 H1p is able to induce higher antibody titers. ELISA assay on coated recombinant HA (H1N1California 2009). Antibody titers were measured on sera from animals immunized with H1 and H1p. Titers were calculated by serial dilution of the sera and represents the dilution giving an OD value three times higher than the background.

Fig. 10 HA (H1N1Mexico2009) pseudotyped virus infection of MDCK cells is more potently neutralized by the serum of animals immunized with H1p. Results of an ELISA assay on coated recombinant HA (H1N1California 2009) with the sera of animals immunized with H1p and NPM1H1p.

Fig. 11 H1p expressed in the context of the triple antigen is able to induce higher antibody titers. ELISA assay on coated recombinant HA (H1N1California 2009). Antibody titers were measured on sera from animals immunized with H1p and NPM1H1p. Titers were calculated by serial dilution of the sera and represent the dilution giving an OD value three times higher than the background.

Fig. 12 Western Blot analysis of NPM1H1p antigen expression in transfected HeLa cells shows that the protein is fully processed. Total lysate of HeLa cells transfected with pNEB-NPM1H1p (Lane 1), with pNEB-NPM1 (Lane 2) and mock transfected (Lane 3). The arrow shows the band corresponding to the fusion protein NPM1 (70kD. A monoclonal anti-NP antibody has been used to detect the intracellular protein.

Fig. 13 H1p derived from processing of NPM1H1p is displayed on the cell membrane and correctly folded. Whole-cell FACS analysis of HeLa cells mock transfected (left panel) or transfected with NPM1H1p (right panel). Cells were incubated with the mouse mAb C179 which binds to a conformational epitope in the HA stem region and then with a secondary anti-mouse antibody PE-conjugated.

Detailed Description of the Invention

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The abbreviations “F” or “F0” are used interchangeably herein and refer to the Fusion protein of paramyxoviruses, preferably of RSV.

The abbreviation “G” refers to the Glycoprotein of paramyxoviruses, preferably of pneumovirinae, more preferably of RSV.

The abbreviation “H” refers to the Hemagglutinin Protein of paramyxoviruses, preferably of morbilliviruses.

The abbreviation “HN” refers to the Hemagglutinin-Neuraminidase Protein of paramyxoviruses, particularly of Respirovirus, Avulavirus and Rubulavirus.

The abbreviation “N” refers to the Nucleocapsid protein of paramyxoviruses, preferably of RSV.

The abbreviation “M” refers to the glycosylated Matrix protein of paramyxoviruses, preferably of RSV.

With respect to paramyxoviruses, the abbreviation “M2” or “M2-1” refers to the non-glycosylated Matrix protein of paramyxoviruses, preferably of RSV.

The abbreviation “P” refers to the Phosphoprotein of paramyxoviruses, preferably of RSV.

With respect to paramyxoviruses, the abbreviation “NS1” and “NS2” refer to the non-structural proteins 1 and 2 of paramyxoviruses, preferably of RSV.

The abbreviation “L” refers to the catalytic subunit of the polymerase of paramyxoviruses, preferably of RSV.

The abrevation “HA” refers to the hemagglutinin of orthomyxovirus, preferably influenzaviruses, more preferably of influenza A virus.

The abrevation “HA0” refers to the precursor protein of hemagglutinin subunits HA1 and HA2 of orthomyxovirus, preferably influenzaviruses, more preferably of influenza A virus

The abrevation “H1p” refers to the modified hemagglutinin of orthomyxovirus, preferably influenzaviruses, more preferably of influenza A virus.

The abrevation “NA” refers to the neuraminidase of orthomyxovirus, preferably influenzaviruses, more preferably of influenza A virus.

The abrevation “NP” refers to the nucleoprotein of orthomyxoviruses, preferably influenzaviruses, more preferably of influenza A virus.

The abrevation "M1" refers to the matrixprotein 1 of orthomyxoviruses, preferably influenzaviruses, more preferably of influenza A virus.

With respect to orthomyxoviruses, the abbreviation "M2" refers to the Matrix protein M2 of orthomyxoviruses, preferably influenzaviruses, more preferably of influenza A virus.

With respect to orthomyxovirus, the abbreviation "NS1" refers to the non-structural protein 1 of orthomyxoviruses, preferably influenzaviruses, more preferably of influenza A virus.

The abbreviation "NS2/NEP" refers to the non-structural protein 2 (also referred to as NEP, nuclear export protein) of orthomyxoviruses, preferably influenzaviruses, more preferably influenza A virus.

The abbreviation "PA" refers to a polymerase subunit protein of orthomyxoviruses, preferably influenzaviruses, more preferably influenza A virus.

The abbreviation "PB1" refers to a polymerase subunit protein of orthomyxoviruses, preferably influenzaviruses, more preferably influenza A virus.

The abbreviation "PB2" refers to a polymerase subunit protein of orthomyxoviruses, preferably influenzaviruses, more preferably influenza A virus.

The abbreviation "PB1-F2" or "PB1F2" refers to a protein encoded by an alternate reading frame in the PB1 Gene segment of orthomyxoviruses, preferably influenzaviruses, more preferably influenza A virus.

The term "expression system" as used herein refers to a system designed to produce one or more gene products of interest. Typically, such system is designed "artificially", i.e. by gene-technological means usable to produce the gene product of interest either *in vitro* in cell-free systems or *in vivo* in cell-based systems. It is understood that naturally occurring expression systems such as for instance native viruses are not encompassed by the expression system of the present invention.

The "gene product of interest" typically refers to a macromolecule such as but not limited to RNA, peptide, polypeptide, or protein, or segment, epitope, or fragment thereof.

In an expression system the gene product of interest is encoded for by one or more nucleic acid molecules. Nucleic acid molecules are understood as a polymeric macromolecules made from nucleotide monomers. Nucleotide monomers are composed of a nucleobase, a five-carbon sugar (such as but not limited to ribose or 2'-deoxyribose), and one to three phosphate groups. Typically, a polynucleotide is formed through phosphodiester bonds between the individual nucleotide monomers. In the context of the present invention referred to nucleic acid molecules include but are not limited to ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The terms "polynucleotide" and "nucleic acid" are used interchangeably herein.

In cell-free expression systems isolated polynucleotides are used as template for *in vitro* translation reactions. In cell-based expression systems polynucleotides are comprised on one or more vectors. As used herein, the term "vector" refers to a protein or a polynucleotide or a mixture thereof which is capable of being introduced or of introducing the proteins and/or nucleic acid comprised therein into a cell. In the context of the present invention it is preferred that the genes of interest encoded by the introduced polynucleotide are expressed within the cell upon introduction of the vector or vectors. Examples of suitable vectors include but are not limited to plasmids, cosmids, phages, viruses or artificial chromosomes.

The phrase “induction of T cell response” refers to the generation or the re-stimulation of virus specific CD4+ or CD8+ T cells. The expression system of the invention can induce or re-stimulate a T cell mediated adaptive response directed to the MHC class I or class II epitopes present in the viral proteins expressed by the polynucleotide. Such T cell response can be measured by art known methods, preferably by *ex-vivo* re-stimulation of T cells with synthetic peptides spanning the entire viral proteins and analysis of proliferation or Interferon-gamma production.

The phrase “induction of B cell response” refers to the generation or the re-stimulation of virus specific B cells producing immunoglobulins of class IgG or IgA. The expression system of the invention can induce or re-stimulate B cells producing antibodies specific for pathogenic, e.g. viral, antigens expressed by the polynucleotide. Such B cell response can be measured by ELISA (Enzyme Linked Immuno Stained Assay) assay with the synthetic antigen of serum or mucosal immunoglobulin. Alternatively the induced antibody titer can be measured by virus neutralization assays.

The phrase “induction of an anti-pathogenic B cell response” refers to the generation or the re-stimulation of virus specific B cells producing immunoglobulins of class IgG or IgA which inactivates, eliminates, blocks and/or neutralizes the respective pathogen such that the disease caused by the pathogen does not break out and/or the symptoms are alleviated. This is also called a “protective immune response” against the pathogen. The expression system of the invention can induce or re-stimulate B cells producing antibodies specific for pathogenic, e.g. viral, antigens expressed by the polynucleotide. Such B cell response can be measured by ELISA (Enzyme Linked Immuno Stained Assay) assay with the synthetic antigen of serum or mucosal immunoglobulin. Alternatively the induced antibody titer can be measured by virus neutralization assays.

The phrase “enhancing an immune response” refers to the strengthening or intensification of the humoral and/or cellular immune response against an immunogen, preferably pathogens, more preferably viruses. The enhancement of the immune response can be measured by

comparing the immune response elicited by an expression system of the invention with the immune response of an expression system expressing the same antigen/immunogen alone by using tests described herein and/or tests well known in the present technical field.

In an expression system, a gene of interest may be encoded by a single polynucleotide or by several separate polynucleotides. In cell-based expression systems one or more polynucleotides may be comprised on a single or on several separate vectors. Each of these polynucleotides may encode the whole or a part of the gene product of interest.

Furthermore, expression systems may encompass "expression control sequences" that regulate the expression of the gene of interest. Typically, expression control sequences are polypeptides or polynucleotides such as but not limited to promoters, enhancers, silencers, insulators, or repressors.

Accordingly, a vector comprising one or more polynucleotides encoding for one or more gene products of interest may comprise further expression control sequences. In a vector comprising more than one polynucleotide encoding for one or more gene products of interest, the expression may be controlled together or separately by one or more expression control sequences. More specifically, each polynucleotide comprised on the vector may be control by a separate expression control sequence or all polynucleotides comprised on the vector may be controlled by a single expression control sequence. Polynucleotides comprised on a single vector controlled by a single expression control sequences preferably form an open reading frame.

The term "expression system" further encompasses the expression of the gene product of interest comprising the transcription of the polynucleotides, RNA splicing, translation into a polypeptide, and post-translational modification of a polypeptide or protein.

The term "open reading frame" (ORF) refers to a sequence of nucleotides, that can be translated into amino acids. Typically, such an ORF contains a start codon, a subsequent region usually having a length which is a multiple of 3 nucleotides, but does not contain a stop codon (TAG, TAA, TGA, UAG, UAA, or UGA) in the given reading frame. Typically, ORFs occur naturally or are constructed artificially, i.e. by gene-technological means. An ORF codes for a protein where the amino acids into which it can be translated form a peptide-linked chain.

The terms "protein", "polypeptide" and "peptide" are used interchangeably herein and refer to any peptide-linked chain of amino acids, regardless of length or post-translational modification.

The term "post-translational" used herein refers to events that occur after the translation of a nucleotide triplet into an amino acid and the formation of a peptide bond to the proceeding amino acid in the sequence. Such post-translational events may occur after the entire polypeptide was formed or already during the translation process on those parts of the polypeptide that have

already been translated. Post-translational events typically alter or modify the chemical or structural properties of the resultant polypeptide. Examples of post-translational events include but are not limited to events such as glycosylation or phosphorylation of amino acids, or cleavage of the peptide chain, e.g. by an endopeptidase.

The term "co-translational" used herein refers to events that occur during the translation process of a nucleotide triplet into an amino acid chain. Those events typically alter or modify the chemical or structural properties of the resultant amino acid chain. Examples of co-translational events include but are not limited to events that may stop the translation process entirely or interrupted the peptide bond formation resulting in two discreet translation products.

As used herein, the terms "polyprotein" or "artificial polyprotein" refer to an amino acid chain that comprises, or essentially consists of or consists of two amino acid chains that are not naturally connected to each other. The polyprotein may comprise one or more further amino acid chains. Each amino acid chain is preferably a complete protein, i.e. spanning an entire ORF, or a fragment, domain or epitope thereof. The individual parts of a polyprotein may either be permanently or temporarily connected to each other. Parts of a polyprotein that are permanently connected are translated from a single ORF and are not later separated co- or post-translationally. Parts of polyproteins that are connected temporarily may also derive from a single ORF but are divided co-translationally due to separation during the translation process or post-translationally due to cleavage of the peptide chain, e.g. by an endopeptidase. Additionally or alternatively, parts of a polyprotein may also be derived from two different ORF and are connected post-translationally, for instance through covalent bonds.

Proteins or polyproteins usable in the present invention (including protein derivatives, protein variants, protein fragments, protein segments, protein epitops and protein domains) can be further modified by chemical modification. This means such a chemically modified polypeptide comprises other chemical groups than the 20 naturally occurring amino acids. Examples of such other chemical groups include without limitation glycosylated amino acids and phosphorylated amino acids. Chemical modifications of a polypeptide may provide advantageous properties as compared to the parent polypeptide, e.g. one or more of enhanced stability, increased biological half-life, or increased water solubility. Chemical modifications applicable to the variants usable in the present invention include without limitation: PEGylation, glycosylation of non-glycosylated parent polypeptides, or the modification of the glycosylation pattern present in the parent polypeptide. Such chemical modifications applicable to the variants usable in the present invention may occur co- or post-translational.

The term "segment" refers to any part of a macromolecule (e.g. a polypeptide, protein or polyprotein) into which this macromolecule can be divided. A macromolecule may consist of

one or more segments. Such segmentation may exist due to functional (e.g. having immunoreactive features or membrane attachment functions) or structural (e.g. nucleotide or amino acid sequence, or secondary or tertiary structure) properties of the macromolecule and/or the individual segment. In the context of the present invention it is preferred that the term "segment" refers to a part of a protein or polyprotein. It is particularly preferred that such segment folds and/or functions independently of the rest of the protein or polyprotein.

An "epitope", also known as antigenic determinant, is the segment of a macromolecule that is recognized by the immune system, specifically by antibodies, B cells, or T cells. Such epitope is that part or segment of a macromolecule capable of binding to an antibody or antigen-binding fragment thereof. In this context, the term "binding" preferably relates to a specific binding. In the context of the present invention it is preferred that the term "epitope" refers to the segment of protein or polyprotein that is recognized by the immune system. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

As used herein, the term "domain" refers to the segment of a protein or polyprotein sequence or structure (or corresponding nucleotide sequence) that can evolve, function, and/or exist independently of the rest of the protein chain. Typically, a protein consists of one or several domains with each of them being three-dimensional structure that are stable and folded independently of the rest of the protein chain. Such domain typically forms an independent functional unit within the protein (e.g. transmembrane-domains, immunoglobulin-like domains, or DNA-binding domains).

As used herein, the term protein or segment "variant" is to be understood as a polypeptide (or segment) which differs in comparison to the polypeptide (or segment, epitop, or domain) from which it is derived by one or more changes in the amino acid sequence. The polypeptide from which a protein variant is derived is also known as the parent polypeptide. Likewise, the segment from which a segment variant is derived from is known as the parent segment. Typically, a variant is constructed artificially, preferably by gene-technological means. Typically, the parent polypeptide is a wild-type protein or wild-type protein domain. In the context of the present invention it is further preferred that a parent polypeptide (or parent segment) is the consensus sequence of two or more wild-type polypeptides (or wild-type segments). Further, the variants usable in the present invention may also be derived from homologs, orthologs, or paralogs of the parent polypeptide or from artificially constructed variant, provided that the variant exhibits at least one biological activity of the parent

polypeptide. The changes in the amino acid sequence may be amino acid exchanges, insertions, deletions, N-terminal truncations, or C-terminal truncations, or any combination of these changes, which may occur at one or several sites. In preferred embodiments, a variant usable in the present invention exhibits a total number of up to 200 (up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200) changes in the amino acid sequence (i.e. exchanges, insertions, deletions, N-terminal truncations, and/or C-terminal truncations). The amino acid exchanges may be conservative and/or non-conservative. In preferred embodiments, a variant usable in the present invention differs from the protein or domain from which it is derived by up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid exchanges, preferably conservative amino acid changes.

Alternatively or additionally, a "variant" as used herein, can be characterized by a certain degree of sequence identity to the parent polypeptide or parent polynucleotide from which it is derived. More precisely, a protein variant in the context of the present invention exhibits at least 80% sequence identity to its parent polypeptide. A polynucleotide variant in the context of the present invention exhibits at least 80% sequence identity to its parent polynucleotide. Preferably, the sequence identity of protein variants is over a continuous stretch of 20, 30, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids. Preferably, the sequence identity of polynucleotide variants is over a continuous stretch of 60, 90, 120, 135, 150, 180, 210, 240, 270, 300 or more nucleotides.

The term "at least 80% sequence identity" is used throughout the specification with regard to polypeptide and polynucleotide sequence comparisons. This expression preferably refers to a sequence identity of at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the respective reference polypeptide or to the respective reference polynucleotide. Preferably, the polypeptide in question and the reference polypeptide exhibit the indicated sequence identity over a continuous stretch of 20, 30, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids or over the entire length of the reference polypeptide. Preferably, the polynucleotide in question and the reference polynucleotide exhibit the indicated sequence identity over a continuous stretch of 60, 90, 120, 135, 150, 180, 210, 240, 270, 300 or more nucleotides or over the entire length of the reference polypeptide.

Variants may additionally or alternatively comprise deletions of amino acids, which may be N-terminal truncations, C-terminal truncations or internal deletions or any combination of these. Such variants comprising N-terminal truncations, C-terminal truncations and/or internal deletions are referred to as "deletion variant" or "fragments" in the context of the present

application. The terms "deletion variant" and "fragment" are used interchangeably herein. A fragment may be naturally occurring (e.g. splice variants) or it may be constructed artificially, preferably by gene-technological means. Preferably, a fragment (or deletion variant) has a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids at its N-terminus and/or at its C-terminus and/or internally as compared to the parent polypeptide, preferably at its N-terminus, at its N- and C-terminus, or at its C-terminus. In case where two sequences are compared and the reference sequence is not specified in comparison to which the sequence identity percentage is to be calculated, the sequence identity is to be calculated with reference to the longer of the two sequences to be compared, if not specifically indicated otherwise. If the reference sequence is indicated, the sequence identity is determined on the basis of the full length of the reference sequence indicated by SEQ ID, if not specifically indicated otherwise. For example, a peptide sequence consisting of 50 amino acids compared to the amino acid sequence of protein F according to SEQ ID NO: 1 may exhibit a maximum sequence identity percentage of 10.04% (50/498) while a sequence with a length of 249 amino acids may exhibit a maximum sequence identity percentage of 50.00% (249/498).

The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via sequence alignments. Such alignments can be carried out with several art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5877), with hmmalign (HMMER package, http://hmmer.wustl.edu/) or with the CLUSTAL algorithm (Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) *Nucleic Acids Res.* 22, 4673-80) available e.g. on http://www.ebi.ac.uk/Tools/clustalw/ or on http://www.ebi.ac.uk/Tools/clustalw2/index.html or on http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html. Preferred parameters used are the default parameters as they are set on http://www.ebi.ac.uk/Tools/clustalw/ or http://www.ebi.ac.uk/Tools/clustalw2/index.html. The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215: 403-410. BLAST polynucleotide searches are performed with the BLASTN program, score = 100, word length = 12, to obtain polynucleotide sequences that are homologous to those nucleic acids which encode F, N, or M2-1. BLAST protein searches are performed with the BLASTP program, score = 50, word length = 3, to obtain amino acid sequences homologous to the F polypeptide, N polypeptide, or M2-1 polypeptide. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402. When utilizing BLAST

and Gapped BLAST programs, the default parameters of the respective programs are used. Sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno M., Bioinformatics 2003b, 19 Suppl 1:I54-I62) or Markov random fields. When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the longer sequence, if not specifically indicated otherwise.

The polynucleotides of the invention encodes proteins, peptides or variants thereof which comprise amino acids which are designated following the standard one- or three-letter code according to WIPO standard ST.25 unless otherwise indicated. If not indicated otherwise, the one- or three letter code is directed at the naturally occuring L-amino acids and the amino acid sequence is indicated in the direction from the N-terminus to the C-terminus of the respective protein, peptide or variant thereof.

"Hybridization" can also be used as a measure of sequence identity or homology between two nucleic acid sequences. A nucleic acid sequence encoding a protein of the invention, or a portion of any of these can be used as a hybridization probe according to standard hybridization techniques. The hybridization of a respective probe to DNA or RNA from a test source is an indication of the presence of the target DNA or RNA, respectively, in the test source. Hybridization conditions are known to those skilled in the art and can be found, for example, in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y., 6.3.1-6.3.6, 1991. "Moderate hybridization conditions" are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1X SSC, 0.1% SDS at 50°C. "Highly stringent conditions" are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by a wash in 0.2 X SSC, 0.1 % SDS at 65°C.

Additionally or alternatively a deletion variant may occur not due to structural deletions of the respective amino acids as described above, but due to these amino acids being inhibited or otherwise not able to fulfill their biological function. Typically, such functional deletion occurs due to the insertions to or exchanges in the amino acid sequence that changes the functional properties of the resultant protein, such as but not limited to alterations in the chemical properties of the resultant protein (i.e. exchange of hydrophobic amino acids to hydrophilic amino acids), alterations in the post-translational modifications of the resultant protein (e.g. post-translational cleavage or glycosylation pattern), or alterations in the secondary or tertiary protein structure. Additionally or alternatively, a functional deletion may also occur due to transcriptional or post-transcriptional gene silencing (e.g. via siRNA) or the presence or absence of inhibitory molecules such as but not limited to protein inhibitors or inhibitory antibodies.

In the context of the present invention it is preferred that a protein (or a segment or a domain or an epitope) being "functionally deleted" refers to the fact that the amino acids or nucleotides of the corresponding sequence are either deleted or present but not fulfilling their biological function.

As used herein, the term "consensus" refers to an amino acid or nucleotide sequence that represents the results of a multiple sequence alignment, wherein related sequences were compared to each other. Such consensus sequence is composed of the amino acids or nucleotides most commonly observed at each position. In the context of the present invention it is preferred that the sequences used in the sequence alignment to obtain the consensus sequence are sequences of different viral subtypes/serotypes strains isolated in various different disease outbreaks worldwide. Each individual sequence used in the sequence alignment is referred to as the sequence of a particular virus "isolate". A more detailed description of the mathematical methods to obtain such consensus is provided in the Example section. In case that for a given position no "consensus nucleotide" or "consensus amino acid" can be determined, e.g. because only two isolates were compared, than it is preferred that the amino acid of each one of the isolates is used. The resulting protein is assessed for its respective B cell and/or T cell inducing ability.

A "peptide linker" (or short: "linker") in the context of the present invention refers to an amino acid sequence of between 1 and 100 amino acids. In preferred embodiments, a peptide linker according to the present invention has a minimum length of at least 1, 2, 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, or 30 amino acids. In further preferred embodiments, a peptide linker according to the present invention has a maximum length of 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, or 15 amino acids or less. It is preferred that peptide linkers provide flexibility among the two amino acid proteins, fragments, segments, epitopes and/or domains that are linked together. Such flexibility is generally increased if the amino acids are small. Thus, preferably the peptide linker of the present invention has an increased content of small amino acids, in particular of glycins, alanines, serines, threonines, leucines and isoleucines. Preferably, more than 20%, 30%, 40%, 50%, 60% or more of the amino acids of the peptide linker are small amino acids. In a preferred embodiment the amino acids of the linker are selected from glycines and serines. In especially preferred embodiments, the above-indicated preferred minimum and maximum lengths of the peptide linker according to the present invention may be combined, if such a combination makes mathematically sense. In further preferred embodiments, the peptide linker of the present invention is non-immunogenic; in particularly preferred embodiments, the peptide linker is non-immunogenic to humans.

The term "cleavage site" as used herein refers to an amino acid sequence or nucleotide sequence where this sequence directs the division, e.g. because it is recognized by a cleaving enzyme, and/or can be divided. Typically, a polypeptide chain is cleaved by hydrolysis of one or more peptide bonds that link the amino acids and a polynucleotide chain is cleaved by hydrolysis of one or more of the phosphodiester bond between the nucleotides. Cleavage of peptide- or phosphodiester-bonds may originate from chemical or enzymatic cleavage. Enzymatic cleavage refers to such cleavage being attained by proteolytic enzymes including but not limited to restriction endonuclease (e.g. type I, type II, type II, type IV or artificial restriction enzymes) and endo- or exo-peptidases or -proteases (e.g. serine-proteases, cysteine-proteases, metallo-proteases, threonine proteases, aspartate proteases, glutamic acid proteases). Typically, enzymatic cleavage occurs due to self-cleavage or is effected by an independent proteolytic enzyme. Enzymatic cleavage of a protein or polypeptide can happen either co- or post-translational. Accordingly, the term "endopeptidase cleavage site" used herein, refers to a cleavage cite within the amino acid or nucleotide sequence where this sequence is cleaved or is cleavable by an endopeptidase (e.g. trypsin, pepsin, elastase, thrombin, collagenase, furin, thermolysin, endopeptidase V8, cathepsins). Alternatively or additionally, the polyprotein of the present invention can be cleaved by an autoprotease, i.e. a protease which cleaves peptide bonds in the same protein molecule which also comprises the protease. Examples of such autoproteases are the NS2 protease from flaviviruses or the VP4 protease of birnaviruses.

Alternatively, the term "cleavage site" refers to an amino acid sequence or nucleotide sequence that prevents the formation of peptide- or phosphodiester-bonds between amino acids or nucleotides, respectively. For instance, the bond formation may be prevented due to co-translational self-processing of the polypeptide or polyprotein resulting in two discontinuous translation products being derived from a single translation event of a single open reading frame. Typically, such self-processing is effected by a "ribosomal skip" caused by a pseudo stop-codon sequence that induces the translation complex to move from one codon to the next without forming a peptide bond. Examples of sequences inducing a ribosomal skip include but are not limited to viral 2A peptides or 2A-like peptide (herein both are collectively referred to as "2A peptide" or interchangeably as "2A site" or "2A cleavage site") which are used by several families of viruses, including Picornavirus, insect viruses, Aphtoviridae, Rotaviruses and Trypanosoma. Best known are 2A sites of rhinovirus and foot-and-mouth disease virus of the Picornaviridae family which are typically used for producing multiple polypeptides from a single ORF.

Accordingly, the term "self-cleavage site" as used herein refers to a cleavage site within the amino acid or nucleotide sequence where this sequence is cleaved or is cleavable without

such cleavage involving any additional molecule or where the peptide- or phosphodiester-bond formation in this sequence is prevented in the first place (e.g. through co-translational self-processing as described above).

It is understood that cleavage sites typically comprise several amino acids or are encoded by several codons (e.g. in those cases, wherein the "cleavage site" is not translated into protein but leads to an interruption of translation). Thus, the cleavage site may also serve the purpose of a peptide linker, i.e. sterically separates two peptides. Thus, in some embodiments a "cleavage site" is both a peptide linker and provides above described cleavage function. In this embodiment the cleavage site may encompass additional N- and/or C-terminal amino acids.

The term "host cell" as used herein refers to a cell that harbours a vector (e.g. a plasmid or virus). Such host cell may either be a prokaryotic (e.g. a bacterial cell) or a eukaryotic cell (e.g. a fungal, plant or animal cell).

"Pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term "carrier", as used herein, refers to a pharmacologically inactive substance such as but not limited to a diluent, excipient, or vehicle with which the therapeutically active ingredient is administered. Such pharmaceutical carriers can be liquid or solid. Liquid carrier include but are not limited to sterile liquids, such as saline solutions in water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously or intranasally by a nebulizer.

Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

The term "composition" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus, in association with it.

The term "adjuvant" refers to agents that augment, stimulate, activate, potentiate, or modulate the immune response to the active ingredient of the composition at either the cellular or humoral level, e.g. immunologic adjuvants stimulate the response of the immune system to the

actual antigen, but have no immunological effect themselves. Examples of such adjuvants include but are not limited to inorganic adjuvants (e.g. inorganic metal salts such as aluminium phosphate or aluminium hydroxide), organic adjuvants (e.g. saponins or squalene), oil-based adjuvants (e.g. Freund's complete adjuvant and Freund's incomplete adjuvant), cytokines (e.g. IL-1β, IL-2, IL-7, IL-12, IL-18, GM-CFS, and INF-γ) particulate adjuvants (e.g. immuno-stimulatory complexes (ISCOMS), liposomes, or biodegradable microspheres), virosomes, bacterial adjuvants (e.g. monophosphoryl lipid A, or muramyl peptides), synthetic adjuvants (e.g. non-ionic block copolymers, muramyl peptide analogues, or synthetic lipid A), or synthetic polynucleotides adjuvants (e.g polyarginine or polylysine).

The term "active ingredient" refers to the substance in a pharmaceutical composition or formulation that is biologically active, i.e. that provides pharmaceutical value. A pharmaceutical composition may comprise one or more active ingredients which may act in conjunction with or independently of each other.

The active ingredient can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as but not limited to those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

As used herein, a "patient" means any mammal, reptile or bird that may benefit from a treatment with a tumour vaccine described herein. Preferably, a "patient" is selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas and human beings. It is particularly preferred that the "patient" is a human being.

As used herein, "treat", "treating" or "treatment" of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in patients that were previously symptomatic for the disorder(s).

As used herein, "prevent", "preventing", "prevention", or "prophylaxis" of a disease or disorder means preventing that such disease or disorder occurs in patient.

As used herein, "administering" includes *in vivo* administration, as well as administration directly to tissue *ex vivo*, such as vein grafts.

An “effective amount” is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to established methods in the art.

Embodiments of the Invention

The present invention will now be further described. In the following passages different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

In a first aspect the invention provides an expression system comprising a first polynucleotide encoding at least one protein, peptide or variant thereof, which induces a T cell response and a second polynucleotide encoding at least one protein, which induces an anti-pathogenic B cell response. One of the advantages provided by the present invention is the fact that the B cell response to the protein, peptide or variant thereof inducing an antipathogenic B cell response can be enhanced, if at the same time a protein inducing a T cell response is administered.

In the context of the present invention the term “expression system” preferably refers to one or more polynucleotide sequences comprising in addition to the first and second polynucleotide the elements to direct transcription and translation of the proteins encoded by the first and second or any further polynucleotide, which may be included in the preferred embodiments outlined below. Such elements included promoter and enhancer elements to direct transcription of mRNA in a cell-free or a cell-based based system, preferably a cell-based system. In another embodiment, wherein the polynucleotides are provided as translatable RNAs is envisioned that the expression system comprises those elements that are necessary for translation and/or stabilization of RNAs encoding the T cell and B cell inducing protein, e.g. polyA-tail, IRES, cap structures etc.

According to a preferred embodiment of the first aspect, the first polynucleotide encodes a protein which induces a reaction of the immune system (i.e. immune response) in a host which is mediated by T cells. A T cell response involves the activation of antigen-specific T

lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells). A T cell response against a protein is induced, if peptides of the protein are processed within the cell and presented to T cells on the surface of the cell via the MHC I or MHC II pathway. Thus, in the context of the present invention preferably those proteins or parts thereof are used for inducing a T cell response that are normally not exposed to, e.g. non structural or internal proteins or parts of structural or internal proteins of a virus not accessible to B-cells.

The second polynucleotide encodes a protein, peptide or variant thereof that induces an anti-pathogenic B cell response. A B cell response is an immune response based on the activation of B lymphocytes, which produce and secrete antigen specific antibodies. B cells involved in such immune response include but are not limited to plasma B cells, memory B cells and B-1 cells. Thus, in the context of the present invention preferably those pathogenic, e.g. viral, proteins or parts thereof are used for inducing a B cell response that are exposed on the outside of the virus, e.g. structural proteins or at least those parts of structural proteins accessible to B-cells on the outside of the pathogen (virus).

In embodiments of the first aspect of the present invention, the first and the second polynucleotide are comprised on separate vectors or on the same vector. Accordingly, the first polynucleotide may be comprised on one vector and the second polynucleotide may be comprised on a second vector. Alternatively or additionally, the first and the second polynucleotide may be comprised on the same vector. It is preferred that the first and the second polynucleotide are comprised on the same vector. It is particularly preferred that the first and the second polynucleotide comprised on the same vector are linked in such that they are expressed as a polyprotein. Preferably, the first and the second polynucleotide form an open reading frame.

It is preferred that the first and the second polynucleotide are expressed as an artificial polyprotein. In the context of the present invention the term "artificial polyprotein" is directed at polyproteins which are not naturally occurring, e.g. which are generated by using recombinant DNA techniques. Accordingly, the proteins, peptides or variants thereof encoded in this artificial polyprotein are preferably derived from pathogens which genome do not encode a polyprotein comprising the proteins, peptides or variants encoded by the first and second polynucleotide (and, optionally, the third polynucleotide) of the invention. Preferably, the first and second polynucleotides are derived from viruses, encoding no polyprotein or a polyprotein wherein the respective polynucleotides have a different order and/or sequence. More preferably, the first and second polynucleotide are derived from a virus which is selected from the group consisting of a DNA virus, a negative sense single stranded (ssRNA(-)) RNA virus or an ambisense RNA virus. Further preferred, the virus is selected from negative-single stranded (ssRNA(-)) RNA virus.

Even more preferred, the virus is selected from enveloped ssRNA(-) viruses, more preferably from the group consisting of paramyxoviruses and orthomyxoviruses.

In preferred embodiments of the first aspect the protein, which induces a T cell response is a non-structural and/or internal protein of a virus, and/or the protein, which induces an anti-pathogenic B cell response is a structural and/or surface protein of a pathogen, preferably a virus, wherein the virus is preferably selected from the group consisting of a DNA virus, a negative-strand RNA virus or an ambisense RNA virus. Even more preferred, the virus is selected from negative-single stranded (ssRNA(-)) RNA virus. Even more preferred, the virus is selected from enveloped ssRNA(-) viruses, more preferably from the group consisting of paramyxoviruses and orthomyxoviruses.

It is preferred that the amino acid sequence of the structural (surface) and/or non-structural (internal) protein comprises consecutive segments or a consensus sequence of one or more different virus isolates.

In the context of the present invention it is preferred that the term "segment" refers to a part of a protein or polyprotein. It is particularly preferred that such segment folds and/or functions independently of the rest of the protein or polyprotein such as but not limited to a domain, an epitope or a fragment thereof. It is understood that a protein variant in the context of the present invention differs in comparison to its parent polypeptide in changes in the amino acid sequence such as amino acid exchanges, insertions, deletions, N-terminal truncations, or C-terminal truncations, or any combination of these changes, which may occur at one or several sites whereby the variant exhibits at least 80% sequence identity to its parent polypeptide.

In preferred embodiments, the structural protein, peptide or a variant thereof is a protein or peptide exposed on the surface of the native pathogen, e.g. a virus. It is preferred that the structural and/or surface protein triggers a T-cell independent immune response such as but not limited to an antibody mediated immune response or an activation of the complement system. In a particularly preferred embodiment, the structural and/or surface protein induces an antibody mediated immune response. Such antibody mediated immune response is based on the activation of B cells which produce and secrete antigen specific antibodies. B cells involved in such immune response include but are not limited to plasma B cells, memory B cells and B-1 cells.

According to a preferred embodiment of the first aspect, the second polynucleotide encodes a protein or variant thereof that induces an anti-pathogenic B cell response. A B cell response is an immune response based on the activation of B lymphocytes, which produce and secrete antigen specific antibodies. B cells involved in such immune response include but are not limited to plasma B cells, memory B cells and B-1 cells. Thus, in the context of the present invention preferably those proteins or parts thereof are used for inducing a B cell response that

are exposed on the outside of a virus, e.g. structural and/or surface proteins or at least those parts of structural and/or surface proteins accessible to B-cells on the outside of a virus. An anti-pathogenic B cell response is a B cell response directed against a pathogen which inactivates, eliminates, blocks and/or neutralizes the respective pathogen such that the disease caused by the pathogen does not break out and/or the sysmptoms are alleviated. In preferred embodiments of the invention, the anti-apthogenic B cell response is effected by antibodies that bind to the surface of a pathogenic organism and attract the first component of the complement cascade with their Fc region and initiate activation of the "classical" complement system. This results in pathogen elimination by two mechanisms. First, the binding of the antibody and complement molecules marks the pathogen for ingestion by phagocytes in a process called opsonization. Secondly, some complement system components form a membrane attack complex to assist antibodies to destroy the pathogen directly. Alternatively, the anti-apthogenic B cell response is effected by antibodies that bind to the pathogen's structural proteins blocking the attachment to cellular receptors. In this way, the antibody can neutralize the infection. As a further aternative, the anti-apthogenic B cell response is effected by antibodies that bind at a specialized region of the pathogen's surface protein, the fusion peptide, which is necessary for the entry of the pathogen into the host cell. The antibody binding results in fixing the protein in a pre-fusion state and blocking infection. The ability of a protein or variant to induce B cell response which is anti-pathogenic can be determined by the skilled person by applying tests and/or assays well known in the art.

In a further preferred embodiment, a membrane attachment domain of the protein exposed on the surface of the native virus or variant thereof is functionally deleted, thus, either being structurally deleted or structurally present but not fulfilling its biological function. In a particularly preferred embodiment, the amino acid sequence corresponding to the membrane attachment domain is deleted. The deletion of the membrane attachment region serves the purpose of ascertaining that the anti-pathogenic B cell response inducing protein is secreted from the cell into which the expression system of the invention has been introduced.

In a further preferred embodiment the anti-pathogenic B cell response inducing protein comprises a secretion signal, which targets the protein to the endoplasmatic reticulum (ER). Such secretion signals are present preferably in the context of a deleted membrane attachment domain. The skilled person is well aware of various such secretion signals, which may be used as heterologous secretion signals, e.g. added to the N-terminus of the anti-pathogenic B cell response inducing protein. Alternatively or additionally a naturally occurring secretion signal may be used, which is, e.g., present in the majority of structural and/or surface viral proteins.

Thus, if naturally present in the respective protein it is preferred that the secretion signal is maintained in a modified version of the structural and/or surface protein.

In embodiments of the first aspect, the non-structural protein is a conserved internal protein suitable for inducing a T cell mediated immune response against the pathogen, preferably the viruses, involving the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells). Thus, preferably the T cell inducing protein of the pathogen (virus) does not comprise a secretion signal.

In the context of the present invention, the protein, peptide or variant thereof encoded by the first polynucleotide is located either N- or C-terminally with respect to the protein, peptide or variant thereof encoded by the second polynucleotide. In a preferred embodiment, the protein, peptide or variant thereof encoded by the second polynucleotide is located C-terminally with respect to the protein, peptide or variant thereof encoded by the first polynucleotide.

Accordingly, embodiments of the present invention have the formula X-Y or Y-X, wherein "X" depicts the T cell response inducing protein and "Y" depicts the anti-pathogenic B cell response inducing protein and a "dash" depicts a peptide bond.

In preferred embodiments of the first aspect, a polynucleotide encoding a cleavage site is positioned between the first polynucleotide and the second polynucleotide. It is within the scope of the present invention that every protein can be combined with any other protein and that any two proteins can or cannot be connected or linked by a cleavage site.

It is preferred that this cleavage site is either a self-cleaving site (i.e. a cleavage site within the amino acid sequence where this sequence is cleaved or is cleavable without such cleavage involving any additional molecule or where the peptide-bond formation in this sequence is prevented in the first place) or an endopeptidase cleavage site (i.e. a cleavage cite within the amino acid sequence where this sequence is cleaved or is cleavable by an endopeptidase, e.g. trypsin, pepsin, elastase, thrombin, collagenase, furin, thermolysin, endopeptidase V8, cathepsins). More preferably, the self-cleaving site is a 2A cleavage site selected from the group consisting of a viral 2A peptide or 2A-like peptide of Picornavirus, insect viruses, Aphtoviridae, Rotaviruses and Trypanosoma, preferably wherein the 2A cleavage site is the 2 A peptide of foot and mouth disease virus. Alternatively or additionally, the polyprotein of the present invention can be cleaved by an autoprotease, i.e. a protease which cleaves peptide bonds in the same protein molecule which also comprises the protease. Examples of such autoproteases are the NS2 protease from flaviviruses or the VP4 protease of birnaviruses

In the context of the present invention, the cleavage site can be positioned N-terminally with respect to the protein, peptide or variant thereof encoded by the first polynucleotide and C-

terminally with respect to the protein, peptide or variant thereof encoded by the second polynucleotide. Alternatively the cleavage site can be positioned C-terminally with respect to the protein, peptide or variant thereof encoded by the first polynucleotide and N-terminally with respect to the protein, peptide or variant thereof encoded by the second polynucleotide.

Accordingly, embodiments of the present invention have the formula X-C-Y or Y-C-X, wherein X" depicts the T cell response inducing protein and "Y" depicts the anti-pathogenic B cell response inducing protein, "C" depicts a cleavage site, and a "dash" depicts a peptide bond.

In a preferred embodiment of the first aspect, the expression system further comprises a third polynucleotide encoding a protein, peptide or a variant thereof of a pathogen.

It is preferred that the protein, peptide or variant thereof encoded by the third polynucleotide is a protein, peptide or variant thereof inducing a T cell response, preferably the third polynucleotide is a protein, peptide or variant thereof which is a non-structural or internal protein, peptide or variant thereof inducing a T cell response.

It is preferred that the protein, peptide or variant thereof encoded by the third polynucleotide differs from the protein, peptide or variant thereof encoded by the first polynucleotide or the second polynucleotide. Preferably, the proteins, peptides or variants thereof encoded by the first, second and the third polynucleotide differ from each other in that they comprise amino acid sequences of different proteins.

In preferred embodiments a polynucleotide encoding a linker is positioned between the second polynucleotide and the third polynucleotide. It is preferred that the linker is a flexible linker, preferably a flexible linker comprising an amino acid sequence according to SEQ ID NO: 6 (Gly-Gly-Gly-Ser-Gly-Gly-Gly).

In preferred embodiments the third polynucleotide is comprised on a separate or on the same vector as the first polynucleotide and/or the second polynucleotide.

Accordingly, the first polynucleotide is comprised on one vector and the second polynucleotide is comprised on a second vector and the third polynucleotide is comprised on a third vector. Alternatively or additionally, the first and the second polynucleotide are comprised on the same vector and the third polynucleotide is comprised on a separate vector, or the first and the third polynucleotide are comprised on the same vector and the second polynucleotide is comprised on a separate vector, or the second and the third polynucleotide are comprised on the same vector and the first polynucleotide is comprised on a separate vector. Alternatively or additionally, the first and the second and the third polynucleotide are comprised on the same vector. It is preferred that the first and the second and the third polynucleotide may be comprised on the same vector. It is particularly preferred that the first and the second and the third polynucleotide comprised on the same vector are linked in such that they are expressed as a

polyprotein. Preferably, the first and the second and the third polynucleotide comprised on the same vector form an open reading frame and, preferably, are expressed as a polyprotein.

In preferred embodiments of this aspect, the protein encoded by the second polynucleotide is located N-terminally with respect to the protein encoded by the first polynucleotide and/or the protein of the optional third polynucleotide, or the protein encoded by the second polynucleotide is located C-terminally with respect to the protein encoded by the first polynucleotide and/or the protein of the optional third polynucleotide.

In even more preferred embodiments of this aspect, the first polynucleotide is located N-terminally with respect to the protein encoded by the second polynucleotide and/or the protein of the optional third polynucleotide is located N-terminally with respect to the protein encoded by the first polynucleotide or is located between the protein encoded by the second polynucleotide and the protein encoded by the first polynucleotide; or the protein encoded by the first polynucleotide is located C-terminally with respect to the protein encoded by the second polynucleotide and/or the protein of the optional third polynucleotide is located C-terminally with respect to the protein encoded by the first polynucleotide or is located between the protein encoded by the second polynucleotide and the protein encoded by the first polynucleotide.

Preferred embodiments of the present invention have the formula X-K-Y, Y-K-X, X-K-Y-Y, Y-Y-K-X, X-Y-K-Y, Y-K-Y-X, X-K-Y-K-Y, Y-K-Y-K-X, X-C-Y, Y-C-X, X-C-Y-Y, Y-Y-C-X, X-Y-C-Y, Y-C-Y-X, X-C-Y-C-Y, Y-C-Y-C-X, X-K-Y-C-Y, Y-C-Y-K-X, X-C-Y-K-Y, or Y-K-Y-C-X, wherein "X" depicts the second polynucleotide encoding at least one protein, peptide or variant thereof, which induces an antipathogenic B cell response and "Y" depicts the first polynucleotide encoding at least one protein, peptide or variant thereof, which induces an T cell response , "K" indicates that one or more peptide linkers are present in this position, "C" indicates that one or more cleavage sites are present in this position and a "dash" depicts a peptide bond. Preferred arrangements are Y-K-Y-C-X or X-C-Y-K-Y.

It is further preferred that the non-structural and/or internal protein encoded by the third polynucleotide is a conserved internal protein suitable for inducing a T cell mediated immune response against the virus involving the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells).

It is within the scope of the present invention that every protein can be combined with any other protein and that any two proteins can or cannot be connected or linked by either a cleavage site or a linker peptide.

In preferred embodiments, the vector or vectors comprising the first, and the second and/or the third polynucleotide is/are selected from the group consisting of plasmid, cosmid,

phage, virus, and artificial chromosome. More preferably, a vector suitable for practicing the present invention is selected from the group consisting of plasmid vectors, cosmid vectors, phage vectors, preferably lambda phage and filamentous phage vectors, viral vectors, adenovirus vectors (e.g., non-replicating Ad5, Ad11, Ad26, Ad35, Ad49, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd 73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, and PanAd3 vectors or replication-competent Ad4 and Ad7 vectors), adeno-associated virus (AAV) vectors (e.g., AAV type 5 and type 2), alphavirus vectors (e.g., Venezuelan equine encephalitis virus (VEE), sindbis virus (SIN), semliki forest virus (SFV), and VEE-SIN chimeras), herpes virus vectors (e.g. vectors derived from cytomegaloviruses, like rhesus cytomegalovirus (RhCMV) (14)), arena virus vectors (e.g. lymphocytic choriomeningitis virus (LCMV) vectors (15)), measles virus vectors, pox virus vectors (e.g., vaccinia virus, modified vaccinia virus Ankara (MVA), NYVAC (derived from the Copenhagen strain of vaccinia), and avipox vectors: canarypox (ALVAC) and fowlpox (FPV) vectors), vesicular stomatitis virus vectors, retrovirus, lentivirus, viral like particles, and bacterial spores. The vectors ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63 and ChAd82 are described in detail in WO 2005/071093. The vectors PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146, and ChAd147 are described in detail in WO 2010/086189. It is particularly preferred that the vector is selected from the group consisting of MVA, ChAd63 and PanAd3.

In preferred embodiments, the expression system is for use in medicine. In more preferred embodiments, the expression system is for use in the prophylaxis or treatment of an infection and/or in the manufacturing of medicament for use in the prophylaxis or treatment of an infection and/or for use in methods of prophylaxis or treatment of an infection, wherein the infection is preferably a viral infection, particularly preferably for use in the prophylaxis or treatment of a pathogen and/or in the manufacturing of medicament for use in the prophylaxis or treatment of a pathogen and/or for use in methods of prophylaxis or treatment of a pathogen, wherein the pathogen preferably is a virus. Preferably, the expression system is for use in the prophylaxis or treatment of an infection by a virus and/or in the manufacturing of medicament for use in the prophylaxis or treatment of a virus and/or for use in methods of prophylaxis or treatment of a virus, wherein the pathogen selected from the group of a DNA virus, a negative-single stranded (ssRNA(-)) RNA virus or an ambisense RNA virus. Further preferred the expression system is for use in the prophylaxis or treatment and/or in the manufacturing of medicament for use in the prophylaxis or treatment of a virus and/or for use in methods of

prophylaxis or treatment of an infection by negative sense single-stranded (ssRNA(-)) RNA virus. Even more preferred, the expression system is for use in the prophylaxis or treatment and/or in the manufacturing of medicament for use in the prophylaxis or treatment of a virus and/or for use in methods of prophylaxis or treatment of an infection by a virus selected from enveloped ssRNA(-) viruses, more preferably from the group consisting of paramyxoviruses and orthomyxoviruses.

In preferred embodiments the expression system is for use in enhancing an immune response. In more preferred embodiments, the expression system is for use in enhancing a B cell immune response against an immunogen, preferably a pathogen, more preferably a virus as defined above.

According to a preferred embodiment of the first aspect, the first polynucleotide encodes a viral protein of a paramyxovirus or variant thereof which induces a reaction of the immune system (i.e. immune response) in a host which is mediated by T cells, and the second polynucleotide encodes a viral protein of a paramyxovirus or variant thereof that induces an anti-pathogenic B cell response against paramyxoviruses. It is preferred that the paramyxovirus whose viral proteins are encoded for by the first and second polynucleotide is selected from the subfamily of Pneumovirinae, Paramyxovirinae, Fer-de-Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, and Murayama-Virus. In even more preferred embodiments, the Pneumovirinae is selected from the group consisting of Pneumovirus, preferably human respiratory syncytial virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV, turkey rinotracheitis virus, and Metapneumovirus, preferably human metapneumovirus (hMPV) and avian metapneumovirus. In even more preferred embodiments, the Paramyxovirinae is selected from the group consisting of Respirovirus, preferably human parainfluenza virus 1 and 3, and Rubulavirus, preferably human parainfluenza virus 2 and 4.

In embodiments of the first aspect of the present invention, the first and the second polynucleotide are comprised on separate vectors or on the same vector. Accordingly, the first polynucleotide may be comprised on one vector and the second polynucleotide may be comprised on a second vector. Alternatively or additionally, the first and the second polynucleotide may be comprised on the same vector. It is preferred that the first and the second polynucleotide are comprised on the same vector. It is particularly preferred that the first and the second polynucleotide comprised on the same vector are linked in such that they are expressed as a polyprotein. Preferably, the first and the second polynucleotide form an open reading frame.

According to preferred embodiments of the first aspect the first polynucleotide encodes a viral protein of a paramyxovirus or variant thereof which induces a reaction of the immune

system (i.e. immune response) in a host which is mediated by T cells. A T cell response involves the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells). A T cell response against a protein is induced, if peptides of the protein are processed within the cell and presented to T cells on the surface of the cell via the MHC I or MHC II pathway. Thus, in the context of the present invention preferably those viral proteins or parts thereof are used for inducing a T cell response that are normally not exposed on the outside of the virus, e.g. non structural or internal proteins or parts of structural or surface proteins not accessible to B-cells on the outside of the virus.

The second polynucleotide encodes a viral protein of a paramyxovirus or variant thereof that induces an anti-pathogenic B cell response against the paramyxovirus. A B cell response is an immune response based on the activation of B lymphocytes, which produce and secrete antigen specific antibodies. B cells involved in such immune response include but are not limited to plasma B cells, memory B cells and B-1 cells. Thus, in the context of the present invention preferably those viral proteins or parts thereof are used for inducing an anti-pathogenic B cell response that are exposed on the outside of the virus, e.g. structural proteins or at least those parts of structural proteins accessible to B-cells on the outside of the virus.

According to a preferred embodiment of the first aspect, the second polynucleotide encodes a viral protein of a paramyxovirus or variant thereof that induces an anti-pathogenic B cell response. A B cell response is an immune response based on the activation of B lymphocytes, which produce and secrete antigen specific antibodies. B cells involved in such immune response include but are not limited to plasma B cells, memory B cells and B-1 cells. Thus, in the context of the present invention preferably those viral proteins or parts thereof are used for inducing an anti-pathogenic B cell response that are exposed on the outside of the virus, e.g. structural and/or surface proteins or at least those parts of structural and/or surface proteins accessible to B-cells on the outside of the virus.

In preferred embodiments of the first aspect the viral protein of a paramyxovirus, which induces a T cell response is a non-structural and/or internal protein of a paramyxovirus, and/or the viral protein of a paramyxovirus, which induces an anti-pathogenic B cell response is a structural and/or surface protein of a paramyxovirus.

It is preferred that the amino acid sequence of the structural (surface) and/or non-structural (internal) protein comprises consecutive segments or a consensus sequence of one or more different paramyxovirus isolates.

In preferred embodiments, the structural protein is a protein exposed on the surface of the native paramyxovirus or a variant thereof. It is preferred that the structural protein triggers a T-

cell independent immune response such as but not limited to an antibody mediated immune response or an activation of the complement system. In a particularly preferred embodiment, the structural and/or surface protein induces an antibody mediated immune response. Such antibody mediated immune response is based on the activation of B cells which produce and secrete antigen specific antibodies. B cells involved in such immune response include but are not limited to plasma B cells, memory B cells and B-1 cells.

In a further preferred embodiment, the membrane attachment domain of the protein exposed on the surface of the native paramyxovirus or variant thereof is functionally deleted, thus, either being structurally deleted or structurally present but not fulfilling its biological function. In a particularly preferred embodiment, the amino acid sequence corresponding to the membrane attachment domain is deleted. The deletion of the membrane attachment region serves the purpose of ascertaining that the anti-pathogenic B cell response inducing protein is secreted from the cell into which the expression system of the invention has been introduced.

In a further preferred embodiment the anti-pathogenic B cell response inducing paramyxovirus protein comprises a secretion signal, which targets the protein to the endoplasmatic reticulum (ER). Thus, if naturally present in the respective structural or surface protein it is preferred that the secretion signal is maintained in a modified version of the structural or surface protein.

It is further preferred that the structural and/or surface protein of the native paramyxovirus is selected from the group consisting of fusion protein (F) and any of the attachment glycoproteins G, H, and HN.

The attachment glycoproteins are found in all enveloped viruses and mediate the initial interaction between the viral envelope and the plasma membrane of the host cell via their binding to carbohydrate moieties or cell adhesion domains of proteins or other molecules on the plasma membrane of the host cell. Thereby, attachment glycoproteins bridge the gap between the virus and the membrane of the host cell. Attachment glycoproteins designated as “H” possess hemagglutinin activity and are found in morbilliviruses and henipaviruses, glycoproteins designated as “HN possess hemagglutinin and neuraminidase activities and are found in respiroviruses, rubulaviruses and avulaviruses. Attachment glycoproteins are designated as “G” when they have neither haemagglutination nor neuraminidase activity. G attachment glycoproteins can be found in all members of Pneumovirinae.

Fusion protein “F” is found in all enveloped viruses and mediates the fusion of the viral envelope with the plasma membrane of the host cell. F is a type I glycoprotein that recognizes receptors present on the cell surface of the host cell to which it binds. F consists of a fusion peptide adjacent to which the transmembrane domains are located, followed by two heptad

repeat (HR) regions, HR1 and HR2, respectively. Upon insertion of the fusion peptide into the plasma membrane of the host cell, the HR1 region forms a trimeric coiled coil structure into whose hydrophobic grooves the HR2 regions folds back. Thereby, a hairpin structure is formed that draws the viral lipid bilayer and cellular plasma membrane even closer together and allows for the formation of a fusion pore and consecutively the complete fusion of both lipid bilayers enabling the virus capsid to enter into the cytoplasm of the host cell. All of these features are common in fusion-mediating proteins of enveloped viruses.

In a preferred embodiment of the first aspect, F comprises, essentially consists of or consists of an amino acid sequence of F of one RSV isolate or a consensus amino acid sequence of two or more different RSV isolates, preferably according to SEQ ID NO: 1, more preferably according to SEQ ID NO: 2 or a variant thereof.

In preferred embodiments of the first aspect, the non-structural protein is a conserved internal protein of paramyxoviruses suitable for inducing a T cell mediated immune response against the paramyxovirus, involving the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells). Thus, preferably the T cell inducing protein of the paramyxovirus does not comprise a secretion signal.

Preferably, the non-structural and/or internal protein is selected from the group consisting of nucleoprotein N, Matrix proteins M and M2, Phosphoprotein P, non structural proteins NS1 and NS2, and the catalytic subunit of the polymerase (L).

The nucleoprotein N serves several functions which include the encapsidation of the RNA genome into a RNAase-resistant nucleocapsid. N also interacts with the M protein during virus assembly and interacts with the P-L polymerase during transcription and replication of the genome.

The matrix protein M is the most abundant protein in paramyxovirus and is considered to be the central organizer of viral morphology by interacting with the cytoplasmatic tail of the integral membrane proteins and the nucleocapsid. M2 is a second membrane-associated protein that is not glycosylated and is mainly found in pneumovirus.

Phosphoprotein P binds to the N and L proteins and forms part of the RNA polymerase complex in all paramyxoviruses. Large protein L is the catalytic subunit of RNA-dependent RNA polymerase.

The function of non-structural proteins NS1 and NS2 has not yet been identified; however, there are indications that they are involved in the viral replication cycle.

In preferred embodiments, N comprises an amino acid sequence of N, of one RSV isolate or a consensus amino acid sequence of two or more different RSV isolates, preferably according

to SEQ ID NO: 3 and wherein M2 comprises an amino acid sequence of M2 of one RSV isolate or a consensus amino acid sequence of two or more different RSV isolates, preferably according to SEQ ID NO: 5. It is further preferred that wherein N comprises the amino acid sequence according to SEQ ID NO: 4 and M2 comprises the amino acid sequence according to SEQ ID NO: 5.

In the context of the present invention, the structural and/or surface protein encoded by the first polynucleotide is located either N- or C-terminally with respect to the non-structural and/or internal protein encoded by the second polynucleotide. In a preferred embodiment, the non-structural and/or internal protein encoded by the second polynucleotide is located C-terminally with respect to the structural and/or surface protein encoded by the first polynucleotide.

More specifically, N, M, M2, P, NS1, NS2, or L can be located N- or C-terminally of F, G, H, or HN. Preferably, N, M, M2, P, NS1, NS2, or L are located C-terminally of F, G, H, or HN. In a more preferred embodiment N or M2 are located C-terminally of F. In a particularly preferred embodiment N is located C-terminally of F.

Accordingly, embodiments of the present invention have the formula X-Y or Y-X, wherein "X" depicts F, G, H, or HN and "Y" depicts N, M, M2, P, NS1, NS2, or L and a "dash" depicts a peptide bond. Preferred arrangements are the following:

F-N, G-N, H-N, HN-N, F-M, G-M, H-M, HN-M, F-M2, G-M2, H-M2, HN-M2, F-P, G-P, H-P, HN-P, F-NS1, G-NS1, H-NS1, HN-NS1, F-NS2, G-NS2, H-NS2, HN-NS2, F-L, G-L, H-L, HN-L, N-F, N-G, N-H, N-HN, M-F, M-G, M-H, M-HN, M2-F, M2-G, M2-H, M2-HN, P-F, P-G, P-H, P-HN, NS1-FF, NS1-G, NS1-H, NS1-HN, NS2-F, NS2-G, NS2-H, NS2-HN, L-F, L-G, L-H, or L-HN.

It is within the scope of the present invention that every protein can be combined with any other protein.

In preferred embodiments of the first aspect, a polynucleotide encoding a cleavage site is positioned between the first polynucleotide and the second polynucleotide.

It is preferred that this cleavage site is either a self-cleaving site (i.e. a cleavage site within the amino acid sequence where this sequence is cleaved or is cleavable without such cleavage involving any additional molecule or where the peptide-bond formation in this sequence is prevented in the first place) or an endopeptidase cleavage site (i.e. a cleavage cite within the amino acid sequence where this sequence is cleaved or is cleavable by an endopeptidase, e.g. trypsin, pepsin, elastase, thrombin, collagenase, furin, thermolysin, endopeptidase V8, cathepsins). More preferably, the self-cleaving site is a 2A cleavage site selected from the group consisting of a viral 2A peptide or 2A-like peptide of Picornavirus,

insect viruses, Aphtoviridae, Rotaviruses and Trypanosoma, preferably wherein the 2A cleavage site is the 2 A peptide of foot and mouth disease virus.

In the context of the present invention, the cleavage site can be positioned N-terminally with respect to the structural and/or surface protein encoded by the first polynucleotide and C-terminally with respect to the non-structural and/or internal protein encoded by the second polynucleotide. Alternatively the cleavage site can be positioned C-terminally with respect to the structural and/or surface protein encoded by the first polynucleotide and N-terminally with respect to the non-structural and/or internal protein encoded by the second polynucleotide. More specifically, the cleavage site can be positioned C- or N-terminally with respect to F, G, H, or HN and C- or N-terminally with respect to N, M, M2, P, NS1, NS2, or L. In a preferred embodiment the cleavage site is located N-terminally with respect to N, M, M2, P, NS1, NS2, or L and C-terminally with respect to F, G, H, or HN. It is particularly preferred that the cleavage site is located N-terminally with respect to N and C-terminally with respect to F.

Accordingly, embodiments of the present invention have the formula X-C-Y or Y-C-X, wherein "X" depicts F, G, H, or HN and "Y" depicts N, M, M2, P, NS1, NS2, or L, "C" depicts a cleavage site, and a "dash" depicts a peptide bond. Preferred arrangements are the following:

F-C-N, G-C-N, H-C-N, HN-C-N, F-C-M, G-C-M, H-C-M, HN-C-M, F-C-M2, G-C-M2, H-C-M2, HN-C-M2, F-C-P, G-C-P, H-C-P, HN-C-P, F-C-NS1, G-C-NS1, H-C-NS1, HN-C-NS1, F-C-NS2, G-C-NS2, H-C-NS2, HN-C-NS2, F-C-L, G-C-L, H-C-L, HN-C-L, N-C-F, N-C-G, N-C-H, N-C-HN, M-C-F, M-C-G, M-C-H, M-C-HN, M2-C-F, M2-C-G, M2-C-H, M2-C-HN, P-C-F, P-C-G, P-C-H, P-C-HN, NS1-C-FF, NS1-C-G, NS1-C-H, NS1-C-HN, NS2-C-F, NS2-C-G, NS2-C-H, NS2-C-HN, L-C-F, L-C-G, L-C-H, or L-C-HN. Particularly, preferred is F-C-N.

It is within the scope of the present invention that every protein can be combined with any other protein and that any two proteins can or cannot be connected or linked by a cleavage site.

In preferred embodiment of the first aspect, the expression system further comprises a third polynucleotide encoding a non-structural and/or internal protein of a paramyxovirus or a variant thereof. Preferably, the non-structural and/or internal protein is of a paramyxovirus selected from the group consisting of Pneumovirinae, Paramyxovirinae, Fer-de-Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, and Murayama-Virus, more preferably, the Pneumovirinae is selected from the group consisting of Pneumovirus, preferably human respiratory syncytical virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV, turkey rinotracheitis and Metapneumovirus, preferably human metapneumovirus, avaian metapneumovirus, more preferably, the Paramyxovirinae is selected from the group consisting of Respirovirus, preferably

human parainfluenza virus 1 and 3, and Rubulavirus, preferably human parainfluenza virus 2 and 4.

In preferred embodiments the third polynucleotide is comprised on a separate or on the same vector as the first polynucleotide and/or the second polynucleotide.

Accordingly, the first polynucleotide is comprised on one vector and the second polynucleotide is comprised on a second vector and the third polynucleotide is comprised on a third vector. Alternatively or additionally, the first and the second polynucleotide are comprised on the same vector and the third polynucleotide is comprised on a separate vector, or the first and the third polynucleotide are comprised on the same vector and the second polynucleotide is comprised on a separate vector, or the second and the third polynucleotide are comprised on the same vector and the first polynucleotide is comprised on a separate vector. Alternatively or additionally, the first and the second and the third polynucleotide are comprised on the same vector. It is preferred that the first and the second and the third polynucleotide may be comprised on the same vector. It is particularly preferred that the first and the second and the third polynucleotide comprised on the same vector are linked in such that they are expressed as a viral polyprotein. Preferably, the first and the second and the third polynucleotide comprised on the same vector form an open reading frame.

It is further preferred that the non-structural and/or internal protein encoded by the third polynucleotide is a conserved internal protein suitable for inducing a T cell mediated immune response against the virus involving the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells).

Preferably, the non-structural and/or internal protein is selected from the group consisting of nucleoprotein N, Matrix proteins M and M2, Phosphoprotein P, non structural proteins NS1 and NS2, and the catalytic subunit of the polymerase (L).

In preferred embodiments, N comprises an amino acid sequence of N, of one RSV isolate or a consensus amino acid sequence of two or more different RSV isolates, preferably according to SEQ ID NO: 3 and wherein M2 comprises an amino acid sequence of M2 of one RSV isolate or a consensus amino acid sequence of two or more different RSV isolates, preferably according to SEQ ID NO: 5. It is further preferred that wherein N comprises the amino acid sequence according to SEQ ID NO: 4 and M2 comprises the amino acid sequence according to SEQ ID NO: 5.

It is preferred that the non-structural and/or internal protein encoded by the third polynucleotide differs from the non-structural and/or internal protein encoded by the second polynucleotide.

The non-structural and/or internal proteins encoded by the second and the third polynucleotide differ from each other in that they comprise amino acid sequences of different viral proteins. For instance, this means that the non-structural and/or internal protein encoded by the second polynucleotide comprises the amino acid sequence of the N protein whilst the non-structural and/or internal protein encoded by the second polynucleotide comprises the amino acid sequence of the M2 protein or vice versa.

The non-structural and/or internal protein encoded by the third polynucleotide can be located either N- or C-terminally of the non-structural and/or internal protein encoded by the second polynucleotide. In a preferred embodiment of the first aspect, the non-structural and/or internal protein encoded by the third polynucleotide is located C-terminally of the non-structural and/or internal protein encoded by the second polynucleotide. More specifically, N or M2 can be located N- or C-terminally of N or M2, preferably N is located C-terminally of M2.

In preferred embodiments a polynucleotide encoding a linker is positioned between the second polynucleotide and the third polynucleotide. It is preferred that the linker is a flexible linker, preferably a flexible linker comprising an amino acid sequence according to SEQ ID NO: 6.

In embodiments of the first aspect, the protein encoded by the second polynucleotide is located N-terminally with respect to the protein encoded by the first polynucleotide and/or the protein of the optional third polynucleotide, or the protein encoded by the second polynucleotide is located C-terminally with respect to the protein encoded by the first polynucleotide and/or the protein of the optional third polynucleotide.

In even more preferred embodiments of this aspect, the first polynucleotide is located N-terminally with respect to the protein encoded by the second polynucleotide and/or the protein of the optional third polynucleotide is located N-terminally with respect to the protein encoded by the first polynucleotide or is located between the protein encoded by the second polynucleotide and the protein encoded by the first polynucleotide; or the protein encoded by the first polynucleotide is located C-terminally with respect to the protein encoded by the second polynucleotide and/or the protein of the optional third polynucleotide is located C-terminally with respect to the protein encoded by the first polynucleotide or is located between the protein encoded by the second polynucleotide and the protein encoded by the first polynucleotide. More specifically, F, G, H, or HN are located C- or N-terminally with respect to N, M, M2, P, NS1, NS2, or L and N, M, M2, P, NS1, NS2, or L are located C- or N-terminally with respect to N or M2. In a preferred embodiment F is located N-terminally with respect to N and M2 is located C-terminally with respect to N.

Accordingly, preferred embodiments of the present invention have the formula X-K-Y, Y-K-X, X-K-Y-Y, Y-Y-K-X, X-Y-K-Y, Y-K-Y-X, X-K-Y-K-Y, Y-K-Y-K-X, X-C-Y, Y-C-X, X-C-Y-Y, Y-Y-C-X, X-Y-C-Y, Y-C-Y-X, X-C-Y-C-Y, Y-C-Y-C-X, X-K-Y-C-Y, Y-C-Y-K-X, X-C-Y-K-Y, or Y-K-Y-C-X, wherein "X" depicts F, G, H, or HN and "Y" depicts N, M, M2, P, NS1, NS2, or L, "K" indicates that one or more peptide linkers are present in this position, "C" indicates that one or more cleavage sites are present in this position and a "dash" depicts a peptide bond. Preferred arrangements are X-C-Y-K-Y. Even more preferred arrangements are the following:

F-K-N, G-K-N, H-K-N, HN-K-N, F-K-M, G-K-M, H-K-M, HN-K-M, F-K-M2, G-K-M2, H-K-M2, HN-K-M2, F-K-P, G-K-P, H-K-P, HN-K-P, F-K-NS1, G-K-NS1, H-K-NS1, HN-K-NS1, F-K-NS2, G-K-NS2, H-K-NS2, HN-K-NS2, F-K-L, G-K-L, H-K-L, HN-K-L, N-K-F, N-K-G, N-K-H, N-K-HN, M-K-F, M-K-G, M-K-H, M-K-HN, M2-K-F, M2-K-G, M2-K-H, M2-K-HN, P-K-F, P-K-G, P-K-H, P-K-HN, NS1-K-FF, NS1-K-G, NS1-K-H, NS1-K-HN, NS2-K-F, NS2-K-G, NS2-K-H, NS2-K-HN, L-K-F, L-K-G, L-K-H, L-K-HN, F-C-N, G-C-N, H-C-N, HN-C-N, F-C-M, G-C-M, H-C-M, HN-C-M, F-C-M2, G-C-M2, H-C-M2, HN-C-M2, F-C-P, G-C-P, H-C-P, HN-C-P, F-C-NS1, G-C-NS1, H-C-NS1, HN-C-NS1, F-C-NS2, G-C-NS2, H-C-NS2, HN-C-NS2, F-C-L, G-C-L, H-C-L, HN-C-L, N-C-F, N-C-G, N-C-H, N-C-HN, M-C-F, M-C-G, M-C-H, M-C-HN, M2-C-F, M2-C-G, M2-C-H, M2-C-HN, P-C-F, P-C-G, P-C-H, P-C-HN, NS1-C-FF, NS1-C-G, NS1-C-H, NS1-C-HN, NS2-C-F, NS2-C-G, NS2-C-H, NS2-C-HN, L-C-F, L-C-G, L-C-H, or L-C-HN, F-N-M, G-N-M, H-N-M, HN-N-M, F-N-M2, G-N-M2, H-N-M2, HN-N-M2, F-N-P, G-N-P, H-N-P, HN-N-P, F-N-NS1, G-N-NS1, H-N-NS1, HN-N-NS1, F-N-NS2, G-N-NS2, H-N-NS2, HN-N-NS2, F-N-L, G-N-L, H-N-L, HN-N-L, F-M-N, G-M-N, H-M-N, HN-M-N, F-M-P, G-M-P, H-M-P, HN-M-P, F-M-NS1, G-M-NS1, H-M-NS1, HN-M-NS1, F-M-NS2, G-M-NS2, H-M-NS2, HN-M-NS2, F-M-L, G-M-L, H-M-L, HN-M-L, F-M2-N, G-M2-N, H-M2-N, HN-M2-N, F-M2-P, G-M2-P, H-M2-P, HN-M2-P, F-M2-NS1, G-M2-NS1, H-M2-NS1, HN-M2-NS1, F-M2-NS2, G-M2-NS2, H-M2-NS2, HN-M2-NS2, F-M2-L, G-M2-L, H-M2-L, HN-M2-L, F-P-N, G-P-N, H-P-N, HN-P-N, F-P-M, G-P-M, H-P-M, HN-P-M, F-P-M2, G-P-M2, H-P-M2, HN-P-M2, F-P-NS1, G-P-NS1, H-P-NS1, HN-P-NS1, F-P-NS2, G-P-NS2, H-P-NS2, HN-P-NS2, F-P-L, G-P-L, H-P-L, HN-P-L, F-NS1-N, G-NS1-N, H-NS1-N, HN-NS1-N, F-NS1-M, G-NS1-M, H-NS1-M, HN-NS1-M, F-NS1-M2, G-NS1-M2, H-NS1-M2, HN-NS1-M2, F-NS1-P, G-NS1-P, H-NS1-P, HN-NS1-P, F-NS1-NS2, G-NS1-NS2, H-NS1-NS2, HN-NS1-NS2, F-NS1-L, G-NS1-L, H-NS1-L, HN-NS1-L, F-NS2-N, G-NS2-N, H-NS2-N, HN-NS2-N, F-NS2-M, G-NS2-M, H-NS2-M, HN-NS2-M, F-NS2-M2, G-NS2-M2, H-NS2-M2, HN-NS2-M2, F-NS2-P, G-NS2-P, H-NS2-P, HN-NS2-P, F-NS2-NS1, G-NS2-NS1, H-NS2-NS1, HN-NS2-NS1, F-NS2-L, G-NS2-L, H-NS2-L, HN-NS2-L, F-L-N, G-L-N, H-L-N, HN-L-

N, F-L-M2, G-L-M2, H-L-M2, HN-L-M2, F-L-P, G-L-P, H-L-P, HN-L-P, F-L-NS1, G-L-NS1, H-L-NS1, HN-L-NS1, F-L-NS2, G-L-NS2, H-L-NS2, HN-L-NS2, M-N-F, M-N-G, M-N-H, M-N-HN, M2-N-F, M2-N-G, M2-N-H, M2-N-HN, P-N-F, P-N-G, P-N-H, P-N-HN, NS1-N-F, NS1-N-G, NS1-N-H, NS1-N-HN, NS2-N-F, NS2-N-G, NS2-N-H, NS2-N-HN, L-N-F, L-N-G, L-N-H, L-N-HN, N-M-F, N-M-G, N-M-H, N-M-HN, P-M-F, P-M-G, P-M-H, P-M-HN, NS1-M-F, NS1-M-G, NS1-M-H, NS1-M-HN, NS2-M-F, NS2-M-G, NS2-M-H, NS2-M-HN, L-M-F, L-M-G, L-M-H, L-M-HN, N-M2-F, N-M2-G, N-M2-H, N-M2-HN, P-M2-F, P-M2-G, P-M2-H, P-M2-HN, NS1-M2-F, NS1-M2-G, NS1-M2-H, NS1-M2-HN, NS2-M2-F, NS2-M2-G, NS2-M2-H, NS2-M2-HN, L-M2-F, L-M2-G, L-M2-H, L-M2-HN, N-P-F, N-P-G, N-P-H, N-P-HN, M-P-F, M-P-G, M-P-H, M-P-HN, M2-P-F, M2-P-G, M2-P-H, M2-P-HN, NS1-P-F, NS1-P-G, NS1-P-H, NS1-P-HN, NS2-P-F, NS2-P-G, NS2-P-H, NS2-P-HN, L-P-F, L-P-G, L-P-H, L-P-HN, N-NS1-F, N-NS1-G, N-NS1-H, N-NS1-HN, M-NS1-F, M-NS1-G, M-NS1-H, M-NS1-HN, M2-NS1-F, M2-NS1-G, M2-NS1-H, M2-NS1-HN, P-NS1-F, P-NS1-G, P-NS1-H, P-NS1-HN, NS2-NS1-F, NS2-NS1-G, NS2-NS1-H, NS2-NS1-HN, L-NS1-F, L-NS1-G, L-NS1-H, L-NS1-HN, N-NS2-F, N-NS2-G, N-NS2-H, N-NS2-HN, M-NS2-F, M-NS2-G, M-NS2-H, M-NS2-HN, M2-NS2-F, M2-NS2-G, M2-NS2-H, M2-NS2-HN, P-NS2-F, P-NS2-G, P-NS2-H, P-NS2-HN, NS1-NS2-F, NS1-NS2-G, NS1-NS2-H, NS1-NS2-HN, L-NS2-F, L-NS2-G, L-NS2-H, L-NS2-HN, N-L-F, N-L-G, N-L-H, N-L-HN, M-L-F, M-L-G, M-L-H, M-L-HN, M2-L-F, M2-L-G, M2-L-H, M2-L-HN, P-L-F, P-L-G, P-L-H, P-L-HN, NS1-L-F, NS1-L-G, NS1-L-H, NS1-L-HN, NS2-L-F, NS2-L-G, NS2-L-H, NS2-L-HN, F-K-N-N, G-K-N-N, H-K-N-N, HN-K-N-N, F-K-N-M, G-K-N-M, H-K-N-M, HN-K-N-M, F-K-N-M2, G-K-N-M2, H-K-N-M2, HN-K-N-M2, F-K-N-P, G-K-N-P, H-K-N-P, HN-K-N-P, F-K-N-NS1, G-K-N-NS1, H-K-N-NS1, HN-K-N-NS1, F-K-N-NS2, G-K-N-NS2, H-K-N-NS2, HN-K-N-NS2, F-K-N-L, G-K-N-L, H-K-N-L, HN-K-N-L, F-C-N-N, G-C-N-N, H-C-N-N, HN-C-N-N, F-C-N-M, G-C-N-M, H-C-N-M, HN-C-N-M, F-C-N-M2, G-C-N-M2, H-C-N-M2, HN-C-N-M2, F-C-N-P, G-C-N-P, H-C-N-P, HN-C-N-P, F-C-N-NS1, G-C-N-NS1, H-C-N-NS1, HN-C-N-NS1, F-C-N-NS2, G-C-N-NS2, H-C-N-NS2, HN-C-N-NS2, F-C-N-L, G-C-N-L, H-C-N-L, HN-C-N-L, F-K-M-M, G-K-M-M, H-K-M-M, HN-K-M-M, F-K-M-M2, G-K-M-M2, H-K-M-M2, HN-K-M-M2, F-K-M-N, G-K-M-N, H-K-M-N, HN-K-M-N, F-K-M-P, G-K-M-P, H-K-M-P, HN-K-M-P, F-K-M-NS1, G-K-M-NS1, H-K-M-NS1, HN-K-M-NS1, F-K-M-NS2, G-K-M-NS2, H-K-M-NS2, HN-K-M-NS2, F-K-M-L, G-K-M-L, H-K-M-L, HN-K-M-L, F-K-M2-M, G-K-M2-M, H-K-M2-M, HN-K-M2-M, F-K-M2-N, G-K-M2-N, H-K-M2-N, HN-K-M2-N, F-K-M2-P, G-K-M2-P, H-K-M2-P, HN-K-M2-P, F-K-M2-NS1, G-K-M2-NS1, H-K-M2-NS1, HN-K-M2-NS1, F-K-M2-NS2, G-K-M2-NS2, H-K-M2-NS2, HN-K-M2-NS2, F-K-M2-L, G-K-M2-L, H-K-M2-L, HN-K-M2-L, F-K-P-N, G-K-P-N, H-K-P-N, HN-K-P-N, F-K-P-M, G-K-P-M, H-K-P-M, HN-K-P-M, F-K-P-M2, G-K-P-M2, H-K-P-

M2, HN-K-P-M2, F-K-P-P, G-K-P-P, H-K-P-P, HN-K-P-P, F-K-P-NS1, G-K-P-NS1, H-K-P-NS1, HN-K-P-NS1, F-K-P-NS2, G-K-P-NS2, H-K-P-NS2, HN-K-P-NS2, F-K-P-L, G-K-P-L, H-K-P-L, HN-K-P-L, F-K-NS1-N, G-K-NS1-N, H-K-NS1-N, HN-K-NS1-N, F-K-NS1-M, G-K-NS1-M, H-K-NS1-M, HN-K-NS1-M, F-K-NS1-M2, G-K-NS1-M2, H-K-NS1-M2, HN-K-NS1-M2, F-K-NS1-P, G-K-NS1-P, H-K-NS1-P, HN-K-NS1-P, F-K-NS1-NS1, G-K-NS1-NS1, H-K-NS1-NS1, HN-K-NS1-NS1, F-K-NS1-NS2, G-K-NS1-NS2, H-K-NS1-NS2, HN-K-NS1-NS2, F-K-NS1-L, G-K-NS1-L, H-K-NS1-L, HN-K-NS1-L, F-K-NS2-N, G-K-NS2-N, H-K-NS2-N, HN-K-NS2-N, F-K-NS2-M, G-K-NS2-M, H-K-NS2-M, HN-K-NS2-M, F-K-NS2-M2, G-K-NS2-M2, H-K-NS2-M2, HN-K-NS2-M2, F-K-NS2-P, G-K-NS2-P, H-K-NS2-P, HN-K-NS2-P, F-K-NS2-NS1, G-K-NS2-NS1, H-K-NS2-NS1, HN-K-NS2-NS1, F-K-NS2-NS2, G-K-NS2-NS2, H-K-NS2-NS2, HN-K-NS2-NS2, F-K-NS2-L, G-K-NS2-L, H-K-NS2-L, HN-K-NS2-L, F-K-L-N, G-K-L-N, H-K-L-N, HN-K-L-N, F-K-L-M, G-K-L-M, H-K-L-M, HN-K-L-M, F-K-L-M2, G-K-L-M2, H-K-L-M2, HN-K-L-M2, F-K-L-P, G-K-L-P, H-K-L-P, HN-K-L-P, F-K-L-NS1, G-K-L-NS1, H-K-L-NS1, HN-K-L-NS1, F-K-L-NS2, G-K-L-NS2, H-K-L-NS2, HN-K-L-NS2, F-K-L-L, G-K-L-L, H-K-L-L, HN-K-L-L, F-N-K-N, G-N-K-N, H-N-K-N, HN-N-K-N, F-N-K-M, G-N-K-M, H-N-K-M, HN-N-K-M, F-N-K-M2, G-N-K-M2, H-N-K-M2, HN-N-K-M2, F-N-K-P, G-N-K-P, H-N-K-P, HN-N-K-P, F-N-K-NS1, G-N-K-NS1, H-N-K-NS1, HN-N-K-NS1, F-N-K-NS2, G-N-K-NS2, H-N-K-NS2, HN-N-K-NS2, F-N-K-L, G-N-K-L, H-N-K-L, HN-N-K-L, F-M-K-N, G-M-K-N, H-M-K-N, HN-M-K-N, F-M-K-M, G-M-K-M, H-M-K-M, HN-M-K-M, F-M-K-M2, G-M-K-M2, H-M-K-M2, HN-M-K-M2, F-M-K-P, G-M-K-P, H-M-K-P HN-M-K-P, F-M-K-NS1, G-M-K-NS1, H-M-K-NS1, HN-M-K-NS1, F-M-K-NS2, G-M-K-NS2, H-M-K-NS2, HN-M-K-NS2, F-M-K-L, G-M-K-L, H-M-K-L, HN-M-K-L, F-M2-K-N, G-M2-K-N, H-M2-K-N, HN-M2-K-N, F-M2-K-M, G-M2-K-M, H-M2-K-M, HN-M2-K-M, F-M2-K-M2, G-M2-K-M2, H-M2-K-M2, HN-M2-K-M2, F-M2-K-P, G-M2-K-P, H-M2-K-P HN-M2-K-P, F-M2-K-NS1, G-M2-K-NS1, H-M2-K-NS1, HN-M2-K-NS1, F-M2-K-NS2, G-M2-K-NS2, H-M2-K-NS2, HN-M2-K-NS2, F-M2-K-L, G-M2-K-L, H-M2-K-L, HN-M2-K-L, F-P-K-N, G-P-K-N, H-P-K-N, HN-P-K-N, F-P-K-M, G-P-K-M, H-P-K-M, HN-P-K-M, F-P-K-M2, G-P-K-M2, H-P-K-M2; HN-P-K-M2, F-P-K-P, G-P-K-P, H-P-K-P, HN-P-K-P, F-P-K-NS1, G-P-K-NS1, H-P-K-NS1, HN-P-K-NS1, F-P-K-NS2, G-P-K-NS2, H-P-K-NS2, HN-P-K-NS2, F-P-K-L, G-P-K-L, H-P-K-L, HN-P-K-L, F-NS1-K-N, G-NS1-K-N, H-NS1-K-N, HN-NS1-K-N, F-NS1-K-M, G-NS1-K-M, H-NS1-K-M; HN-NS1-K-M, F-NS1-K-M2, G-NS1-K-M2, H-NS1-K-M2; HN-NS1-K-M2, F-NS1-K-P, G-NS1-K-P, H-NS1-K-P, HN-NS1-K-P, F-NS1-K-NS1, G-NS1-K-NS1, H-NS1-K-NS1, HN-NS1-K-NS1, F-NS1-K-NS2, G-NS1-K-NS2, H-NS1-K-NS2, HN-NS1-K-NS2, F-NS1-K-L, G-NS1-K-L, H-NS1-K-L, HN-NS1-K-L, F-NS2-K-N, G-NS2-K-N, H-NS2-K-N, HN-NS2-K-N, F-NS2-K-M, G-NS2-K-M, H-NS2-K-M, HN-NS2-K-M, F-NS2-K-

M2, G-NS2-K-M2, H-NS2-K-M2, HN-NS2-K-M2, F-NS2-K-P, G-NS2-K-P, H-NS2-K-P, HN-NS2-K-P, F-NS2-K-NS1, G-NS2-K-NS1, H-NS2-K-NS1, HN-NS2-K-NS1, F-NS2-K-NS2, G-NS2-K-NS2, H-NS2-K-NS2, HN-NS2-K-NS2, F-NS2-K-L, G-NS2-K-L, H-NS2-K-L, HN-NS2-K-L, F-C-M-M, G-C-M-M, H-C-M-M, HN-C-M-M, F-C-M-M2, G-C-M-M2, H-C-M-M2, HN-C-M-M2, F-C-M-N, G-C-M-N, H-C-M-N, HN-C-M-N, F-C-M-P, G-C-M-P, H-C-M-P, HN-C-M-P, F-C-M-NS1, G-C-M-NS1, H-C-M-NS1, HN-C-M-NS1, F-C-M-NS2, G-C-M-NS2, H-C-M-NS2, HN-C-M-NS2, F-C-M-L, G-C-M-L, H-C-M-L, HN-C-M-L, F-C-M2-M, G-C-M2-M, H-C-M2-M, HN-C-M2-M, F-C-M2-N, G-C-M2-N, H-C-M2-N, HN-C-M2-N, F-C-M2-P, G-C-M2-P, H-C-M2-P, HN-C-M2-P, F-C-M2-NS1, G-C-M2-NS1, H-C-M2-NS1, HN-C-M2-NS1, F-C-M2-NS2, G-C-M2-NS2, H-C-M2-NS2, HN-C-M2-NS2, F-C-M2-L, G-C-M2-L, H-C-M2-L, HN-C-M2-L, F-C-P-N, G-C-P-N, H-C-P-N, HN-C-P-N, F-C-P-M, G-C-P-M, H-C-P-M, HN-C-P-M, F-C-P-M2, G-C-P-M2, H-C-P-M2, HN-C-P-M2, F-C-P-P, G-C-P-P, H-C-P-P, HN-C-P-P, F-C-P-NS1, G-C-P-NS1, H-C-P-NS1, HN-C-P-NS1, F-C-P-NS2, G-C-P-NS2, H-C-P-NS2, HN-C-P-NS2, F-C-P-L, G-C-P-L, H-C-P-L, HN-C-P-L, F-C-NS1-N, G-C-NS1-N, H-C-NS1-N, HN-C-NS1-N, F-C-NS1-M, G-C-NS1-M, H-C-NS1-M, HN-C-NS1-M, F-C-NS1-M2, G-C-NS1-M2, H-C-NS1-M2, HN-C-NS1-M2, F-C-NS1-P, G-C-NS1-P, H-C-NS1-P, HN-C-NS1-P, F-C-NS1-NS1, G-C-NS1-NS1, H-C-NS1-NS1, HN-C-NS1-NS1, F-C-NS1-NS2, G-C-NS1-NS2, H-C-NS1-NS2, HN-C-NS1-NS2, F-C-NS1-L, G-C-NS1-L, H-C-NS1-L, HN-C-NS1-L, F-C-NS2-N, G-C-NS2-N, H-C-NS2-N, HN-C-NS2-N, F-C-NS2-M, G-C-NS2-M, H-C-NS2-M, HN-C-NS2-M, F-C-NS2-M2, G-C-NS2-M2, H-C-NS2-M2, HN-C-NS2-M2, F-C-NS2-P, G-C-NS2-P, H-C-NS2-P, HN-C-NS2-P, F-C-NS2-NS1, G-C-NS2-NS1, H-C-NS2-NS1, HN-C-NS2-NS1, F-C-NS2-NS2, G-C-NS2-NS2, H-C-NS2-NS2, HN-C-NS2-NS2, F-C-NS2-L, G-C-NS2-L, H-C-NS2-L, HN-C-NS2-L, F-C-L-N, G-C-L-N, H-C-L-N, HN-C-L-N, F-C-L-M, G-C-L-M, H-C-L-M, HN-C-L-M, F-C-L-M2, G-C-L-M2, H-C-L-M2, HN-C-L-M2, F-C-L-P, G-C-L-P, H-C-L-P, HN-C-L-P, F-C-L-NS1, G-C-L-NS1, H-C-L-NS1, HN-C-L-NS1, F-C-L-NS2, G-C-L-NS2, H-C-L-NS2, HN-C-L-NS2, F-C-L-L, G-C-L-L, H-C-L-L, HN-C-L-L, F-N-C-N, G-N-C-N, H-N-C-N, HN-N-C-N, F-N-C-M, G-N-C-M, H-N-C-M, HN-N-C-M, F-N-C-M2, G-N-C-M2, H-N-C-M2, HN-N-C-M2, F-N-C-P, G-N-C-P, H-N-C-P, HN-N-C-P, F-N-C-NS1, G-N-C-NS1, H-N-C-NS1, HN-N-C-NS1, F-N-C-NS2, G-N-C-NS2, H-N-C-NS2, HN-N-C-NS2, F-N-C-L, G-N-C-L, H-N-C-L, HN-N-C-L, F-M-C-N, G-M-C-N, H-M-C-N, HN-M-C-N, F-M-C-M, G-M-C-M, H-M-C-M, HN-M-C-M, F-M-C-M2, G-M-C-M2, H-M-C-M2, HN-M-C-M2, F-M-C-P, G-M-C-P, H-M-C-P HN-M-C-P, F-M-C-NS1, G-M-C-NS1, H-M-C-NS1, HN-M-C-NS1, F-M-C-NS2, G-M-C-NS2, H-M-C-NS2, HN-M-C-NS2, F-M-C-L, G-M-C-L, H-M-C-L, HN-M-C-L, F-M2-C-N, G-M2-C-N, H-M2-C-N, HN-M2-C-N, F-M2-C-M, G-M2-C-M, H-M2-C-M, HN-M2-C-M, F-M2-C-M2, G-M2-C-M2, H-M2-C-M2, HN-M2-C-M2, F-M2-C-P, G-M2-C-P, H-M2-C-P HN-M2-

C-P, F-M2-C-NS1, G-M2-C-NS1, H-M2-C-NS1, HN-M2-C-NS1, F-M2-C-NS2, G-M2-C-NS2, H-M2-C-NS2, HN-M2-C-NS2, F-M2-C-L, G-M2-C-L, H-M2-C-L, HN-M2-C-L, F-P-C-N, G-P-C-N, H-P-C-N, HN-P-C-N, F-P-C-M, G-P-C-M, H-P-C-M, HN-P-C-M, F-P-C-M2, G-P-C-M2, H-P-C-M2; HN-P-C-M2, F-P-C-P, G-P-C-P, H-P-C-P, HN-P-C-P, F-P-C-NS1, G-P-C-NS1, H-P-C-NS1, HN-P-C-NS1, F-P-C-NS2, G-P-C-NS2, H-P-C-NS2, HN-P-C-NS2, F-P-C-L, G-P-C-L, H-P-C-L, HN-P-C-L, F-NS1-C-N, G-NS1-C-N, H-NS1-C-N, HN-NS1-C-N, F-NS1-C-M, G-NS1-C-M, H-NS1-C-M; HN-NS1-C-M, F-NS1-C-M2, G-NS1-C-M2, H-NS1-C-M2; HN-NS1-C-M2, F-NS1-C-P, G-NS1-C-P, H-NS1-C-P, HN-NS1-C-P, F-NS1-C-NS1, G-NS1-C-NS1, H-NS1-C-NS1, HN-NS1-C-NS1, F-NS1-C-NS2, G-NS1-C-NS2, H-NS1-C-NS2, HN-NS1-C-NS2, F-NS1-C-L, G-NS1-C-L, H-NS1-C-L, HN-NS1-C-L, F-NS2-C-N, G-NS2-C-N, H-NS2-C-N, HN-NS2-C-N, F-NS2-C-M, G-NS2-C-M, H-NS2-C-M, HN-NS2-C-M, F-NS2-C-M2, G-NS2-C-M2, H-NS2-C-M2, HN-NS2-C-M2, F-NS2-C-P, G-NS2-C-P, H-NS2-C-P, HN-NS2-C-P, F-NS2-C-NS1, G-NS2-C-NS1, H-NS2-C-NS1, HN-NS2-C-NS1, F-NS2-C-NS2, G-NS2-C-NS2, H-NS2-C-NS2, HN-NS2-C-NS2, F-NS2-C-L, G-NS2-C-L, H-NS2-C-L, HN-NS2-C-L, F-L-K-N, G-L-K-N, H-L-K-N, HN-L-K-N, F-L-K-M, G-L-K-M, H-L-K-M, HN-L-K-M, F-L-K-M2, G-L-K-M2, H-L-K-M2, HN-L-K-M2, F-L-K-P, G-L-K-P, H-L-K-P, HN-L-K-P, F-L-K-NS1, G-L-K-NS1, H-L-K-NS1, HN-L-K-NS1, F-L-K-NS2, G-L-K-NS2, H-L-K-NS2, HN-L-K-NS2, F-L-K-L, G-L-K-L, H-L-K-L, HN-L-K-L, F-L-C-N, G-L-C-N, H-L-C-N, HN-L-C-N, F-L-C-M, G-L-C-M, H-L-C-M, HN-L-C-M, F-L-C-M2, G-L-C-M2, H-L-C-M2, HN-L-C-M2, F-L-C-P, G-L-C-P, H-L-C-P, HN-L-C-P, F-L-C-NS1, G-L-C-NS1, H-L-C-NS1, HN-L-C-NS1, F-L-C-NS2, G-L-C-NS2, H-L-C-NS2, HN-L-C-NS2, F-L-C-L, G-L-C-L, H-L-C-L, HN-L-C-L, F-K-N-K-N, G-K-N-K-N, H-K-N-K-N, HN-K-N-K-N, F-K-M-K-N, G-K-M-K-N, H-K-M-K-N, HN-K-M-K-N, F-K-M2-K-N, G-K-M2-K-N, H-K-M2-K-N, HN-K-M2-K-N, F-K-P-K-N, G-K-P-K-N, H-K-P-K-N, HN-K-P-K-N, F-K-NS1-K-N, G-K-NS1-K-N, H-K-NS1-K-N, HN-K-NS1-K-N, F-K-NS2-K-N, G-K-NS2-K-N, H-K-NS2-K-N, HN-K-NS2-K-N, F-K-L-K-N, G-K-L-K-N, H-K-L-K-N, HN-K-L-K-N, F-K-N-K-M, G-K-N-K-M, H-K-N-K-M, HN-K-N-K-M, F-K-M-K-M, G-K-M-K-M, H-K-M-K-M, HN-K-M-K-M, F-K-M2-K-M, G-K-M2-K-M, H-K-M2-K-M, HN-K-M2-K-M, F-K-P-K-M, G-K-P-K-M, H-K-P-K-M, HN-K-P-K-M, F-K-NS1-K-M, G-K-NS1-K-M, H-K-NS1-K-M, HN-K-NS1-K-M, F-K-NS2-K-M, G-K-NS2-K-M, H-K-NS2-K-M, HN-K-NS2-K-M, F-K-L-K-M, G-K-L-K-M, H-K-L-K-M, HN-K-L-K-M, F-K-N-K-M2, G-K-N-K-M2, H-K-N-K-M2, HN-K-N-K-M2, F-K-M-K-M2, G-K-M-K-M2, H-K-M-K-M2, HN-K-M-K-M2, F-K-M2-K-M2, G-K-M2-K-M2, H-K-M2-K-M2, HN-K-M2-K-M2, F-K-P-K-M2, G-K-P-K-M2, H-K-P-K-M2, HN-K-P-K-M2, F-K-NS1-K-M2, G-K-NS1-K-M2, H-K-NS1-K-M2, HN-K-NS1-K-M2, F-K-NS2-K-M2, G-K-NS2-K-M2, H-K-NS2-K-M2, HN-K-NS2-K-M2, F-K-L-K-M2, G-K-L-K-M2, H-K-L-K-M2, HN-K-L-K-M2, F-K-N-K-P, G-K-N-K-P, H-K-N-K-P, HN-K-N-

K-P, F-K-M-K-P, G-K-M-K-P, H-K-M-K-P, HN-K-M-K-P, F-K-M2-K-P, G-K-M2-K-P, H-K-M2-K-P, HN-K-M2-K-P, F-K-P-K-P, G-K-P-K-P, H-K-P-K-P, HN-K-P-K-P, F-K-NS1-K-P, G-K-NS1-K-P, H-K-NS1-K-P, HN-K-NS1-K-P, F-K-NS2-K-P, G-K-NS2-K-P, H-K-NS2-K-P, HN-K-NS2-K-P, F-K-L-K-P, G-K-L-K-P, H-K-L-K-P, HN-K-L-K-P, F-K-N-K-NS1, G-K-N-K-NS1, H-K-N-K-NS1, HN-K-N-K-NS1, F-K-M-K-NS1, G-K-M-K-NS1, H-K-M-K-NS1, HN-K-M-K-NS1, F-K-M2-K-NS1, G-K-M2-K-NS1, H-K-M2-K-NS1, HN-K-M2-K-NS1, F-K-P-K-NS1, G-K-P-K-NS1, H-K-P-K-NS1, HN-K-P-K-NS1, F-K-NS1-K-NS1, G-K-NS1-K-NS1, H-K-NS1-K-NS1, HN-K-NS1-K-NS1, F-K-NS2-K-NS1, G-K-NS2-K-NS1, H-K-NS2-K-NS1, HN-K-NS2-K-NS1, F-K-L-K-NS1, G-K-L-K-NS1, H-K-L-K-NS1, HN-K-L-K-NS1, F-K-N-K-NS2, G-K-N-K-NS2, H-K-N-K-NS2, HN-K-N-K-NS2, F-K-M-K-NS2, G-K-M-K-NS2, H-K-M-K-NS2, HN-K-M-K-NS2, F-K-M2-K-NS2, G-K-M2-K-NS2, H-K-M2-K-NS2, HN-K-M2-K-NS2, F-K-P-K-NS2, G-K-P-K-NS2, H-K-P-K-NS2, HN-K-P-K-NS2, F-K-NS1-K-NS2 G-K-NS1-K-NS2, H-K-NS1-K-NS2, HN-K-NS1-K-NS2, F-K-NS2-K-NS2 G-K-NS2-K-NS2, H-K-NS2-K-NS2, HN-K-NS2-K-NS2, F-K-L-K-NS2, G-K-L-K-NS2, H-K-L-K-NS2, HN-K-L-K-NS2, F-K-N-K-L, G-K-N-K-L, H-K-N-K-L, HN-K-N-K-K, F-K-M-K-L, G-K-M-K-L, H-K-M-K-L, HN-K-M-K-L, F-K-M2-K-L, G-K-M2-K-L, H-K-M2-K-L, HN-K-M2-K-L, F-K-P-K-L, G-K-P-K-L, H-K-P-K-L, HN-K-P-K-L, F-K-NS1-K-L, G-K-NS1-K-L, H-K-NS1-K-L, HN-K-NS1-K-L, F-K-NS2-K-L, G-K-NS2-K-L, H-K-NS2-K-L, HN-K-NS2-K-L, F-K-L-K-L, G-K-L-K-L, H-K-L-K-L, HN-K-L-K-L, N-K-N-F, N-K-N-G, N-K-N-H, N-K-N-HN, M-K-N-F, M-K-N-G, M-K-N-H, M-K-N-HN, M2-K-N-F, M2-K-N-G, M2-K-N-H, M2-K-N-HN, P-K-N-F, P-K-N-G, P-K-N-H, P-K-N-HN, NS1-K-N-F, NS1-K-N-G, NS1-K-N-H, NS1-K-N-HN, NS2-K-N-F, NS2-K-N-G, NS2-K-N-H, NS2-K-N-HN, L-K-N-F, L-K-N-G, L-K-N-H, L-K-N-HN, N-K-M-F, N-K-M-G, N-K-M-H, N-K-M-HN, M-K-M-F, M-K-M-G, M-K-M-H, M-K-M-HN, M2-K-M-F, M2-K-M-G, M2-K-M-H, M2-K-M-HN, P-K-M-F, P-K-M-G, P-K-M-H, P-K-M-HN, NS1-K-M-F, NS1-K-M-G, NS1-K-M-H, NS1-K-M-HN, NS2-K-M-F, NS2-K-M-G, NS2-K-M-H, NS2-K-M-HN, L-K-M-F, L-K-M-G, L-K-M-H, L-K-M-HN, N-K-M2-F, N-K-M2-G, N-K-M2-H, N-K-M2-HN, M-K-M2-F, M-K-M2-G, M-K-M2-H, M-K-M2-HN, M2-K-M2-F, M2-K-M2-G, M2-K-M2-H, M2-K-M2-HN, P-K-M2-F, P-K-M2-G, P-K-M2-H, P-K-M2-HN, NS1-K-M2-F, NS1-K-M2-G, NS1-K-M2-H, NS1-K-M2-HN, NS2-K-M2-F, NS2-K-M2-G, NS2-K-M2-H, NS2-K-M2-HN, L-K-M2-F, L-K-M2-G, L-K-M2-H, L-K-M2-HN, N-K-P-F, N-K-P-G, N-K-P-H, N-K-P-HN, M-K-P-F, M-K-P-G, M-K-P-H, M-K-P-HN, M2-K-P-F, M2-K-P-G, M2-K-P-H, M2-K-P-HN, P-K-P-F, P-K-P-G, P-K-P-H, P-K-P-HN, NS1-K-P-F, NS1-K-P-G, NS1-K-P-H, NS1-K-P-HN, NS2-K-P-F, NS2-K-P-G, NS2-K-P-H, NS2-K-P-HN, L-K-P-F, L-K-P-G, L-K-P-H, L-K-P-HN, N-K-NS1-F, N-K-NS1-G, N-K-NS1-H, N-K-NS1-HN, M-K-NS1-F, M-K-NS1-G, M-K-NS1-H, M-K-NS1-HN, M2-K-NS1-F, M2-K-NS1-G, M2-K-NS1-H, M2-K-NS1-HN, P-K-NS1-

F, P-K-NS1-G, P-K-NS1-H, P-K-NS1-HN, NS1-K-NS1-F, NS1-K-NS1-G, NS1-K-NS1-H, NS1-K-NS1-HN, NS2-K-NS1-F, NS2-K-NS1-G, NS2-K-NS1-H, NS2-K-NS1-HN, L-K-NS1-F, L-K-NS1-G, L-K-NS1-H, L-K-NS1-HN,N-K-NS2-F, N-K-NS2-G, N-K-NS2-H, N-K-NS2-HN, M-K-NS2-F, M-K-NS2-G, M-K-NS2-H, M-K-NS2-HN, M2-K-NS2-F, M2-K-NS2-G, M2-K-NS2-H, M2-K-NS2-HN, P-K-NS2-F, P-K-NS2-G, P-K-NS2-H, P-K-NS2-HN, NS1-K-NS2-F, NS1-K-NS2-G, NS1-K-NS2-H, NS1-K-NS2-HN, NS2-K-NS2-F, NS2-K-NS2-G, NS2-K-NS2-H, NS2-K-NS2-HN, L-K-NS2-F, L-K-NS2-G, L-K-NS2-H, L-K-NS2-HN, N-K-L-F, N-K-L-G, N-K-L-H, N-K-L-HN, M-K-L-F, M-K-L-G, M-K-L2-H, M-K-L-HN, M2-K-L-F, M2-K-L-G, M2-K-L-H, M2-K-L-HN, P-K-L-F, P-K-L-G, P-K-L-H, P-K-L-HN, NS1-K-L-F, NS1-K-L-G, NS1-K-L-H, NS1-K-L-HN, NS2-K-L-F, NS2-K-L-G, NS2-K-L-H, NS2-K-L-HN, L-K-L-F, L-K-L-G, L-K-L-H, L-K-L-HN, N-N-K-F, N-N-K-G, N-N-K-H, N-N-K-HN, N-M-K-F, N-M-K-G, N-M-K-H, N-M-K-HN, N-M2-K-F, N-M2-K-G, N-M2-K-H, N-M2-K-HN, N-P-K-F, N-P-K-G, N-P-K-H, N-P-K-HN, N-NS1-K-F, N-NS1-K-G, N-NS1-K-H, N-NS1-K-HN, N-NS2-K-F, N-NS2-K-G, N-NS2-K-H, N-NS2-K-HN, N-L-K-F, N-L-K-G, N-L-K-H, N-L-K-HN, M-N-K-F, M-N-K-G, M-N-K-H, M-N-K-HN, M-M-K-F, M-M-K-G, M-M-K-H, M-M-K-HN, M-M2-K-F, M-M2-K-G, M-M2-K-H, M-M2-K-HN, M-P-K-F, M-P-K-G, M-P-K-H, M-P-K-HN, M-NS1-K-F, M-NS1-K-G, M-NS1-K-H, M-NS1-K-HN, M-NS2-K-F, M-NS2-K-G, M-NS2-K-H, M-NS2-K-HN, M-L-K-F, M-L-K-G, M-L-K-H, M-L-K-HN, M2-N-K-F, M2-N-K-G, M2-N-K-H, M2-N-K-HN, M2-M-K-F, M2-M-K-G, M2-M-K-H, M2-M-K-HN, M2-M2-K-F, M2-M2-K-G, M2-M2-K-H, M2-M2-K-HN, M2-P-K-F, M2-P-K-G, M2-P-K-H, M2-P-K-HN, M2-NS1-K-F, M2-NS1-K-G, M2-NS1-K-H, M2-NS1-K-HN, M2-NS2-K-F, M2-NS2-K-G, M2-NS2-K-H, M2-NS2-K-HN, M2-L-K-F, M2-L-K-G, M2-L-K-H, M2-L-K-HN, P-N-K-F, P-N-K-G, P-N-K-H, P-N-K-HN, P-M-K-F, P-M-K-G, P-M-K-H, P-M-K-HN, P-M2-K-F, P-M2-K-G, P-M2-K-H, P-M2-K-HN, P-P-K-F, P-P-K-G, P-P-K-H, P-P-K-HN, P-NS1-K-F, P-NS1-K-G, P-NS1-K-H, P-NS1-K-HN, P-NS2-K-F, P-NS2-K-G, P-NS2-K-H, P-NS2-K-HN, P-L-K-F, P-L-K-G, P-L-K-H, P-L-K-HN, NS1-N-K-F, NS1-N-K-G, NS1-N-K-H, NS1-N-K-HN, NS1-M-K-F, NS1-M-K-G, NS1-M-K-H, NS1-M-K-HN, NS1-M2-K-F, NS1-M2-K-G, NS1-M2-K-H, NS1-M2-K-HN, NS1-P-K-F, NS1-P-K-G, NS1-P-K-H, NS1-P-K-HN, NS1-NS1-K-F, NS1-NS1-K-G, NS1-NS1-K-H, NS1-NS1-K-HN, NS1-NS2-K-F, NS1-NS2-K-G, NS1-NS2-K-H, NS1-NS2-K-HN, NS1-L-K-F, NS1-L-K-G, NS1-L-K-H, NS1-L-K-HN, NS2-N-K-F, NS2-N-K-G, NS2-N-K-H, NS2-N-K-HN, NS2-M-K-F, NS2-M-K-G, NS2-M-K-H, NS2-M-K-HN, NS2-M2-K-F, NS2-M2-K-G, NS2-M2-K-H, NS2-M2-K-HN, NS2-P-K-F, NS2-P-K-G, NS2-P-K-H, NS2-P-K-HN, NS2-NS1-K-F, NS2-NS1-K-G, NS2-NS1-K-H, NS2-NS1-K-HN, NS2-NS2-K-F, NS2-NS2-K-G, NS2-NS2-K-H, NS2-NS2-K-HN, NS2-L-K-F, NS2-L-K-G, NS2-L-K-H, NS2-L-K-HN, L-N-K-F, L-N-K-G, L-N-K-H, L-N-K-HN, L-M-K-F, L-M-K-G, L-M-K-H, L-M-K-HN, L-M2-K-F, L-M2-

K-G, L-M2-K-H, L-M2-K-HN, L-L-K-F, L-P-K-G, L-P-K-H, L-P-K-HN, L-NS1-K-F, L-NS1-K-G, L-NS1-K-H, L-NS1-K-HN, L-NS2-K-F, L-NS2-K-G, L-NS2-K-H, L-NS2-K-HN, L-L-K-F, L-L-K-G, L-L-K-H, L-L-K-HN, N-K-N-K-F, N-K-N-K-G, N-K-N-K-H, N-K-N-K-HN, N-K-M-K-F, N-K-M-K-G, N-K-M-K-H, N-K-M-K-HN, N-K-M2-K-F, N-K-M2-K-G, N-K-M2-K-H, N-K-M2-K-HN, N-K-P-K-F, N-K-P-K-G, N-K-P-K-H, N-K-P-K-HN, N-K-NS1-K-F, N-K-NS1-K-G, N-K-NS1-K-H, N-K-NS1-K-HN, N-K-NS2-K-F, N-K-NS2-K-G, N-K-NS2-K-H, N-K-NS2-K-HN, N-K-L-K-F, N-K-L-K-G, N-K-L-K-H, N-K-L-K-HN, M-K-N-K-F, M-K-N-K-G, M-K-N-K-H, M-K-N-K-HN, M-K-M-K-F, M-K-M-K-G, M-K-M-K-H, M-K-M-K-HN, M-K-M2-K-F, M-K-M2-K-G, M-K-M2-K-H, M-K-M2-K-HN, M-K-P-K-F, M-K-P-K-G, M-K-P-K-H, M-K-P-K-HN, M-K-NS1-K-F, M-K-NS1-K-G, M-K-NS1-K-H, M-K-NS1-K-HN, M-K-NS2-K-F, M-K-NS2-K-G, M-K-NS2-K-H, M-K-NS2-K-HN, M-K-L-K-F, M-K-L-K-G, M-K-L-K-H, M-K-L-K-HN, M2-K-N-K-F, M2-K-N-K-G, M2-K-N-K-H, M2-K-N-K-HN, M2-K-M-K-F, M2-K-M-K-G, M2-K-M-K-H, M2-K-M-K-HN, M2-K-M2-K-F, M2-K-M2-K-G, M2-K-M2-K-H, M2-K-M2-K-HN, M2-K-P-K-F, M2-K-P-K-G, M2-K-P-K-H, M2-K-P-K-HN, M2-K-NS1-K-F, M2-K-NS1-K-G, M2-K-NS1-K-H, M2-K-NS1-K-HN, M2-K-NS2-K-F, M2-K-NS2-K-G, M2-K-NS2-K-H, M2-K-NS2-K-HN, M2-K-L-K-F, M2-K-L-K-G, M2-K-L-K-H, M2-K-L-K-HN, P-K-N-K-F, P-K-N-K-G, P-K-N-K-H, P-K-N-K-HN, P-K-M-K-F, P-K-M-K-G, P-K-M-K-H, P-K-M-K-HN, P-K-M2-K-F, P-K-M2-K-G, P-K-M2-K-H, P-K-M2-K-HN, P-K-P-K-F, P-K-P-K-G, P-K-P-K-H, P-K-P-K-HN, P-K-NS1-K-F, P-K-NS1-K-G, P-K-NS1-K-H, P-K-NS1-K-HN, P-K-NS2-K-F, P-K-NS2-K-G, P-K-NS2-K-H, P-K-NS2-K-HN, P-K-L-K-F, P-K-L-K-G, P-K-L-K-H, P-K-L-K-HN, NS1-K-N-K-F, NS1-K-N-K-G, NS1-K-N-K-H, NS1-K-N-K-HN, NS1-K-M-K-F, NS1-K-M-K-G, NS1-K-M-K-H, NS1-K-M-K-HN, NS1-K-M2-K-F, NS1-K-M2-K-G, NS1-K-M2-K-H, NS1-K-M2-K-HN, NS1-K-P-K-F, NS1-K-P-K-G, NS1-K-P-K-H, NS1-K-P-K-HN, NS1-K-NS1-K-F, NS1-K-NS1-K-G, NS1-K-NS1-K-H, NS1-K-NS1-K-HN, NS1-K-NS2-K-F, NS1-K-NS2-K-G, NS1-K-NS2-K-H, NS1-K-NS2-K-HN, NS1-K-L-K-F, NS1-K-L-K-G, NS1-K-L-K-H, NS1-K-L-K-HN, NS2-K-N-K-F, NS2-K-N-K-G, NS2-K-N-K-H, NS2-K-N-K-HN, NS2-K-M-K-F, NS2-K-M-K-G, NS2-K-M-K-H, NS2-K-M-K-HN, NS2-K-M2-K-F, NS2-K-M2-K-G, NS2-K-M2-K-H, NS2-K-M2-K-HN, NS2-K-P-K-F, NS2-K-P-K-G, NS2-K-P-K-H, NS2-K-P-K-HN, NS2-K-NS1-K-F, NS2-K-NS1-K-G, NS2-K-NS1-K-H, NS2-K-NS1-K-HN, NS2-K-NS2-K-F, NS2-K-NS2-K-G, NS2-K-NS2-K-H, NS2-K-NS2-K-HN, NS2-K-L-K-F, NS2-K-L-K-G, NS2-K-L-K-H, NS2-K-L-K-HN, L-K-N-K-F, L-K-N-K-G, L-K-N-K-H, L-K-N-K-HN, L-K-M-K-F, L-K-M-K-G, L-K-M-K-H, L-K-M-K-HN, L-K-M2-K-F, L-K-M2-K-G, L-K-M2-K-H, L-K-M2-K-HN, L-K-P-K-F, L-K-P-K-G, L-K-P-K-H, L-K-P-K-HN, L-K-NS1-K-F, L-K-NS1-K-G, L-K-NS1-K-H, L-K-NS1-K-HN, L-K-NS2-K-F, L-K-NS2-K-G, L-K-NS2-K-H, L-K-NS2-K-HN, L-K-L-K-F, L-K-L-K-G, L-K-L-K-H, or L-K-L-K-HN, F-C-N-

K-N, G-C-N-K-N, H-C-N-K-N, HN-C-N-K-N, F-C-M-K-N, G-C-M-K-N, H-C-M-K-N, HN-C-M-K-N, F-C-M2-K-N, G-C-M2-K-N, H-C-M2-K-N, HN-C-M2-K-N, F-C-P-K-N, G-C-P-K-N, H-C-P-K-N, HN-C-P-K-N, F-C-NS1-K-N, G-C-NS1-K-N, H-C-NS1-K-N, HN-C-NS1-K-N, F-C-NS2-K-N, G-C-NS2-K-N, H-C-NS2-K-N, HN-C-NS2-K-N, F-C-L-K-N, G-C-L-K-N, H-C-L-K-N, HN-C-L-K-N, F-C-N-K-M, G-C-N-K-M, H-C-N-K-M, HN-C-N-K-M, F-C-M-K-M, G-C-M-K-M, H-C-M-K-M, HN-C-M-K-M, F-C-M2-K-M, G-C-M2-K-M, H-C-M2-K-M, HN-C-M2-K-M, F-C-P-K-M, G-C-P-K-M, H-C-P-K-M, HN-C-P-K-M, F-C-NS1-K-M, G-C-NS1-K-M, H-C-NS1-K-M, HN-C-NS1-K-M, F-C-NS2-K-M, G-C-NS2-K-M, H-C-NS2-K-M, HN-C-NS2-K-M, F-C-L-K-M, G-C-L-K-M, H-C-L-K-M, HN-C-L-K-M, F-C-N-K-M2, G-C-N-K-M2, H-C-N-K-M2, HN-C-N-K-M2, F-C-M-K-M2, G-C-M-K-M2, H-C-M-K-M2, HN-C-M-K-M2, F-C-M2-K-M2, G-C-M2-K-M2, H-C-M2-K-M2, HN-C-M2-K-M2, F-C-P-K-M2, G-C-P-K-M2, H-C-P-K-M2, HN-C-P-K-M2, F-C-NS1-K-M2, G-C-NS1-K-M2, H-C-NS1-K-M2, HN-C-NS1-K-M2, F-C-NS2-K-M2, G-C-NS2-K-M2, H-C-NS2-K-M2, HN-C-NS2-K-M2, F-C-L-K-M2, G-C-L-K-M2, H-C-L-K-M2, HN-C-L-K-M2, F-C-N-K-P, G-C-N-K-P, H-C-N-K-P, HN-C-N-K-P, F-C-M-K-P, G-C-M-K-P, H-C-M-K-P, HN-C-M-K-P, F-C-M2-K-P, G-C-M2-K-P, H-C-M2-K-P, HN-C-M2-K-P, F-C-P-K-P, G-C-P-K-P, H-C-P-K-P, HN-C-P-K-P, F-C-NS1-K-P, G-C-NS1-K-P, H-C-NS1-K-P, HN-C-NS1-K-P, F-C-NS2-K-P, G-C-NS2-K-P, H-C-NS2-K-P, HN-C-NS2-K-P, F-C-L-K-P, G-C-L-K-P, H-C-L-K-P, HN-C-L-K-P, F-C-N-K-NS1, G-C-N-K-NS1, H-C-N-K-NS1, HN-C-N-K-NS1, F-C-M-K-NS1, G-C-M-K-NS1, H-C-M-K-NS1, HN-C-M-K-NS1, F-C-M2-K-NS1, G-C-M2-K-NS1, H-C-M2-K-NS1, HN-C-M2-K-NS1, F-C-P-K-NS1, G-C-P-K-NS1, H-C-P-K-NS1, HN-C-P-K-NS1, F-C-NS1-K-NS1, G-C-NS1-K-NS1, H-C-NS1-K-NS1, HN-C-NS1-K-NS1, F-C-NS2-K-NS1, G-C-NS2-K-NS1, H-C-NS2-K-NS1, HN-C-NS2-K-NS1, F-C-L-K-NS1, G-C-L-K-NS1, H-C-L-K-NS1, HN-C-L-K-NS1, F-C-N-K-NS2, G-C-N-K-NS2, H-C-N-K-NS2, HN-C-N-K-NS2, F-C-M-K-NS2, G-C-M-K-NS2, H-C-M-K-NS2, HN-C-M-K-NS2, F-C-M2-K-NS2, G-C-M2-K-NS2, H-C-M2-K-NS2, HN-C-M2-K-NS2, F-C-P-K-NS2, G-C-P-K-NS2, H-C-P-K-NS2, HN-C-P-K-NS2, F-C-NS1-K-NS2 G-C-NS1-K-NS2, H-C-NS1-K-NS2, HN-C-NS1-K-NS2, F-C-NS2-K-NS2 G-C-NS2-K-NS2, H-C-NS2-K-NS2, HN-C-NS2-K-NS2, F-C-L-K-NS2, G-C-L-K-NS2, H-C-L-K-NS2, HN-C-L-K-NS2, F-C-N-K-L, G-C-N-K-L, H-C-N-K-L, HN-C-N-K-L, F-C-M-K-L, G-C-M-K-L, H-C-M-K-L, HN-C-M-K-L, F-C-M2-K-L, G-C-M2-K-L, H-C-M2-K-L, HN-C-M2-K-L, F-C-P-K-L, G-C-P-K-L, H-C-P-K-L, HN-C-P-K-L, F-C-NS1-K-L, G-C-NS1-K-L, H-C-NS1-K-L, HN-C-NS1-K-L, F-C-NS2-K-L, G-C-NS2-K-L, H-C-NS2-K-L, HN-C-NS2-K-L, F-C-L-K-L, G-C-L-K-L, H-C-L-K-L, HN-C-L-K-L, N-C-N-F, N-C-N-G, N-C-N-H, N-C-N-HN, M-C-N-F, M-C-N-G, M-C-N-H, M-C-N-HN, M2-C-N-F, M2-C-N-G, M2-C-N-H, M2-C-N-HN, P-C-N-F, P-C-N-G, P-C-N-H, P-C-N-HN, NS1-C-N-F, NS1-C-N-G, NS1-C-N-H, NS1-C-N-HN, NS2-C-N-F, NS2-C-N-G, NS2-C-N-H, NS2-C-N-HN,

L-C-N-F, L-C-N-G, L-C-N-H, L-C-N-HN, N-C-M-F, N-C-M-G, N-C-M-H, N-C-M-HN, M-C-M-F, M-C-M-G, M-C-M-H, M-C-M-HN, M2-C-M-F, M2-C-M-G, M2-C-M-H, M2-C-M-HN, P-C-M-F, P-C-M-G, P-C-M-H, P-C-M-HN, NS1-C-M-F, NS1-C-M-G, NS1-C-M-H, NS1-C-M-HN, NS2-C-M-F, NS2-C-M-G, NS2-C-M-H, NS2-C-M-HN, L-C-M-F, L-C-M-G, L-C-M-H, L-C-M-HN, N-C-M2-F, N-C-M2-G, N-C-M2-H, N-C-M2-HN, M-C-M2-F, M-C-M2-G, M-C-M2-H, M-C-M2-HN, M2-C-M2-F, M2-C-M2-G, M2-C-M2-H, M2-C-M2-HN, P-C-M2-F, P-C-M2-G, P-C-M2-H, P-C-M2-HN, NS1-C-M2-F, NS1-C-M2-G, NS1-C-M2-H, NS1-C-M2-HN, NS2-C-M2-F, NS2-C-M2-G, NS2-C-M2-H, NS2-C-M2-HN, L-C-M2-F, L-C-M2-G, L-C-M2-H, L-C-M2-HN, N-C-P-F, N-C-P-G, N-C-P-H, N-C-P-HN, M-C-P-F, M-C-P-G, M-C-P-H, M-C-P-HN, M2-C-P-F, M2-C-P-G, M2-C-P-H, M2-C-P-HN, P-C-P-F, P-C-P-G, P-C-P-H, P-C-P-HN, NS1-C-P-F, NS1-C-P-G, NS1-C-P-H, NS1-C-P-HN, NS2-C-P-F, NS2-C-P-G, NS2-C-P-H, NS2-C-P-HN, L-C-P-F, L-C-P-G, L-C-P-H, L-C-P-HN, N-C-NS1-F, N-C-NS1-G, N-C-NS1-H, N-C-NS1-HN, M-C-NS1-F, M-C-NS1-G, M-C-NS1-H, M-C-NS1-HN, M2-C-NS1-F, M2-C-NS1-G, M2-C-NS1-H, M2-C-NS1-HN, P-C-NS1-F, P-C-NS1-G, P-C-NS1-H, P-C-NS1-HN, NS1-C-NS1-F, NS1-C-NS1-G, NS1-C-NS1-H, NS1-C-NS1-HN, NS2-C-NS1-F, NS2-C-NS1-G, NS2-C-NS1-H, NS2-C-NS1-HN, L-C-NS1-F, L-C-NS1-G, L-C-NS1-H, L-C-NS1-HN, N-C-NS2-F, N-C-NS2-G, N-C-NS2-H, N-C-NS2-HN, M-C-NS2-F, M-C-NS2-G, M-C-NS2-H, M-C-NS2-HN, M2-C-NS2-F, M2-C-NS2-G, M2-C-NS2-H, M2-C-NS2-HN, P-C-NS2-F, P-C-NS2-G, P-C-NS2-H, P-C-NS2-HN, NS1-C-NS2-F, NS1-C-NS2-G, NS1-C-NS2-H, NS1-C-NS2-HN, NS2-C-NS2-F, NS2-C-NS2-G, NS2-C-NS2-H, NS2-C-NS2-HN, L-C-NS2-F, L-C-NS2-G, L-C-NS2-H, L-C-NS2-HN, N-C-L-F, N-C-L-G, N-C-L-H, N-C-L-HN, M-C-L-F, M-C-L-G, M-C-L2-H, M-C-L-HN, M2-C-L-F, M2-C-L-G, M2-C-L-H, M2-C-L-HN, P-C-L-F, P-C-L-G, P-C-L-H, P-C-L-HN, NS1-C-L-F, NS1-C-L-G, NS1-C-L-H, NS1-C-L-HN, NS2-C-L-F, NS2-C-L-G, NS2-C-L-H, NS2-C-L-HN, L-C-L-F, L-C-L-G, L-C-L-H, L-C-L-HN, N-N-C-F, N-N-C-G, N-N-C-H, N-N-C-HN, N-M-C-F, N-M-C-G, N-M-C-H, N-M-C-HN, N-M2-C-F, N-M2-C-G, N-M2-C-H, N-M2-C-HN, N-P-C-F, N-P-C-G, N-P-C-H, N-P-C-HN, N-NS1-C-F, N-NS1-C-G, N-NS1-C-H, N-NS1-C-HN, N-NS2-C-F, N-NS2-C-G, N-NS2-C-H, N-NS2-C-HN, N-L-C-F, N-L-C-G, N-L-C-H, N-L-C-HN, M-N-C-F, M-N-C-G, M-N-C-H, M-N-C-HN, M-M-C-F, M-M-C-G, M-M-C-H, M-M-C-HN, M-M2-C-F, M-M2-C-G, M-M2-C-H, M-M2-C-HN, M-P-C-F, M-P-C-G, M-P-C-H, M-P-C-HN, M-NS1-C-F, M-NS1-C-G, M-NS1-C-H, M-NS1-C-HN, M-NS2-C-F, M-NS2-C-G, M-NS2-C-H, M-NS2-C-HN, M-L-C-F, M-L-C-G, M-L-C-H, M-L-C-HN, M2-N-C-F, M2-N-C-G, M2-N-C-H, M2-N-C-HN, M2-M-C-F, M2-M-C-G, M2-M-C-H, M2-M-C-HN, M2-M2-C-F, M2-M2-C-G, M2-M2-C-H, M2-M2-C-HN, M2-P-C-F, M2-P-C-G, M2-P-C-H, M2-P-C-HN, M2-NS1-C-F, M2-NS1-C-G, M2-NS1-C-H, M2-NS1-C-HN, M2-NS2-C-F, M2-NS2-C-G, M2-NS2-C-H, M2-NS2-C-HN, M2-L-C-F, M2-L-C-G, M2-L-C-H, M2-L-C-HN, P-N-C-F,

P-N-C-G, P-N-C-H, P-N-C-HN, P-M-C-F, P-M-C-G, P-M-C-H, P-M-C-HN, P-M2-C-F, P-M2-C-G, P-M2-C-H, P-M2-C-HN, P-P-C-F, P-P-C-G, P-P-C-H, P-P-C-HN, P-NS1-C-F, P-NS1-C-G, P-NS1-C-H, P-NS1-C-HN, P-NS2-C-F, P-NS2-C-G, P-NS2-C-H, P-NS2-C-HN, P-L-C-F, P-L-C-G, P-L-C-H, P-L-C-HN, NS1-N-C-F, NS1-N-C-G, NS1-N-C-H, NS1-N-C-HN, NS1-M-C-F, NS1-M-C-G, NS1-M-C-H, NS1-M-C-HN, NS1-M2-C-F, NS1-M2-C-G, NS1-M2-C-H, NS1-M2-C-HN, NS1-P-C-F, NS1-P-C-G, NS1-P-C-H, NS1-P-C-HN, NS1-NS1-C-F, NS1-NS1-C-G, NS1-NS1-C-H, NS1-NS1-C-HN, NS1-NS2-C-F, NS1-NS2-C-G, NS1-NS2-C-H, NS1-NS2-C-HN, NS1-L-C-F, NS1-L-C-G, NS1-L-C-H, NS1-L-C-HN, NS2-N-C-F, NS2-N-C-G, NS2-N-C-H, NS2-N-C-HN, NS2-M-C-F, NS2-M-C-G, NS2-M-C-H, NS2-M-C-HN, NS2-M2-C-F, NS2-M2-C-G, NS2-M2-C-H, NS2-M2-C-HN, NS2-P-C-F, NS2-P-C-G, NS2-P-C-H, NS2-P-C-HN, NS2-NS1-C-F, NS2-NS1-C-G, NS2-NS1-C-H, NS2-NS1-C-HN, NS2-NS2-C-F, NS2-NS2-C-G, NS2-NS2-C-H, NS2-NS2-C-HN, NS2-L-C-F, NS2-L-C-G, NS2-L-C-H, NS2-L-C-HN, L-N-C-F, L-N-C-G, L-N-C-H, L-N-C-HN, L-M-C-F, L-M-C-G, L-M-C-H, L-M-C-HN, L-M2-C-F, L-M2-C-G, L-M2-C-H, L-M2-C-HN, L-L-C-F, L-P-C-G, L-P-C-H, L-P-C-HN, L-NS1-C-F, L-NS1-C-G, L-NS1-C-H, L-NS1-C-HN, L-NS2-C-F, L-NS2-C-G, L-NS2-C-H, L-NS2-C-HN, L-L-C-F, L-L-C-G, L-L-C-H, L-L-C-HN, N-C-N-K-F, N-C-N-K-G, N-C-N-K-H, N-C-N-K-HN, N-C-M-K-F, N-C-M-K-G, N-C-M-K-H, N-C-M-K-HN, N-C-M2-K-F, N-C-M2-K-G, N-C-M2-K-H, N-C-M2-K-HN, N-C-P-K-F, N-C-P-K-G, N-C-P-K-H, N-C-P-K-HN, N-C-NS1-K-F, N-C-NS1-K-G, N-C-NS1-K-H, N-C-NS1-K-HN, N-C-NS2-K-F, N-C-NS2-K-G, N-C-NS2-K-H, N-C-NS2-K-HN, N-C-L-K-F, N-C-L-K-G, N-C-L-K-H, N-C-L-K-HN, M-C-N-K-F, M-C-N-K-G, M-C-N-K-H, M-C-N-K-HN, M-C-M-K-F, M-C-M-K-G, M-C-M-K-H, M-C-M-K-HN, M-C-M2-K-F, M-C-M2-K-G, M-C-M2-K-H, M-C-M2-K-HN, M-C-P-K-F, M-C-P-K-G, M-C-P-K-H, M-C-P-K-HN, M-C-NS1-K-F, M-C-NS1-K-G, M-C-NS1-K-H, M-C-NS1-K-HN, M-C-NS2-K-F, M-C-NS2-K-G, M-C-NS2-K-H, M-C-NS2-K-HN, M-C-L-K-F, M-C-L-K-G, M-C-L-K-H, M-C-L-K-HN, M2-C-N-K-F, M2-C-N-K-G, M2-C-N-K-H, M2-C-N-K-HN, M2-C-M-K-F, M2-C-M-K-G, M2-C-M-K-H, M2-C-M-K-HN, M2-C-M2-K-F, M2-C-M2-K-G, M2-C-M2-K-H, M2-C-M2-K-HN, M2-C-P-K-F, M2-C-P-K-G, M2-C-P-K-H, M2-C-P-K-HN, M2-C-NS1-K-F, M2-C-NS1-K-G, M2-C-NS1-K-H, M2-C-NS1-K-HN, M2-C-NS2-K-F, M2-C-NS2-K-G, M2-C-NS2-K-H, M2-C-NS2-K-HN, M2-C-L-K-F, M2-C-L-K-G, M2-C-L-K-H, M2-C-L-K-HN, P-C-N-K-F, P-C-N-K-G, P-C-N-K-H, P-C-N-K-HN, P-C-M-K-F, P-C-M-K-G, P-C-M-K-H, P-C-M-K-HN, P-C-M2-K-F, P-C-M2-K-G, P-C-M2-K-H, P-C-M2-K-HN, P-C-P-K-F, P-C-P-K-G, P-C-P-K-H, P-C-P-K-HN, P-C-NS1-K-F, P-C-NS1-K-G, P-C-NS1-K-H, P-C-NS1-K-HN, P-C-NS2-K-F, P-C-NS2-K-G, P-C-NS2-K-H, P-C-NS2-K-HN, P-C-L-K-F, P-C-L-K-G, P-C-L-K-H, P-C-L-K-HN, NS1-C-N-K-F, NS1-C-N-K-G, NS1-C-N-K-H, NS1-C-N-K-HN, NS1-C-M-K-F, NS1-C-M-K-G, NS1-C-M-K-H, NS1-C-M-K-HN, NS1-C-M2-K-F, NS1-C-M2-K-G, NS1-C-M2-K-

H, NS1-C-M2-K-HN, NS1-C-P-K-F, NS1-C-P-K-G, NS1-C-P-K-H, NS1-C-P-K-HN, NS1-C-NS1-K-F, NS1-C-NS1-K-G, NS1-C-NS1-K-H, NS1-C-NS1-K-HN, NS1-C-NS2-K-F, NS1-C-NS2-K-G, NS1-C-NS2-K-H, NS1-C-NS2-K-HN, NS1-C-L-K-F, NS1-C-L-K-G, NS1-C-L-K-H, NS1-C-L-K-HN, NS2-C-N-K-F, NS2-C-N-K-G, NS2-C-N-K-H, NS2-C-N-K-HN, NS2-C-M-K-F, NS2-C-M-K-G, NS2-C-M-K-H, NS2-C-M-K-HN, NS2-C-M2-K-F, NS2-C-M2-K-G, NS2-C-M2-K-H, NS2-C-M2-K-HN, NS2-C-P-K-F, NS2-C-P-K-G, NS2-C-P-K-H, NS2-C-P-K-HN, NS2-C-NS1-K-F, NS2-C-NS1-K-G, NS2-C-NS1-K-H, NS2-C-NS1-K-HN, NS2-C-NS2-K-F, NS2-C-NS2-K-G, NS2-C-NS2-K-H, NS2-C-NS2-K-HN, NS2-C-L-K-F, NS2-C-L-K-G, NS2-C-L-K-H, NS2-C-L-K-HN, L-C-N-K-F, L-C-N-K-G, L-C-N-K-H, L-C-N-K-HN, L-C-M-K-F, L-C-M-K-G, L-C-M-K-H, L-C-M-K-HN, L-C-M2-K-F, L-C-M2-K-G, L-C-M2-K-H, L-C-M2-K-HN, L-C-P-K-F, L-C-P-K-G, L-C-P-K-H, L-C-P-K-HN, L-C-NS1-K-F, L-C-NS1-K-G, L-C-NS1-K-H, L-C-NS1-K-HN, L-C-NS2-K-F, L-C-NS2-K-G, L-C-NS2-K-H, L-C-NS2-K-HN, L-C-L-K-F, L-C-L-K-G, L-C-L-K-H, or L-C-L-K-HN. Most preferably the arrangement is F-C-N-K-M2.

In preferred embodiments, the expression system is for use in the prophylaxis or treatment of viral infection, particularly preferably for use in the prophylaxis or treatment of a paramyxovirus infection, preferably a RSV infection and/or in the manufacturing of medicament for use in the prophylaxis or treatment of a paramyxovirus infection, preferably a RSV infection, and/or for use in methods of prophylaxis or treatment of an of a paramyxovirus infection, preferably a RSV infection.

In preferred embodiments, the expression system is for use in enhancing an immune response, preferably a B cell immune response against a paramyxovirus infection, preferably a RSV infection.

According to a preferred embodiment of the first aspect, the first polynucleotide encodes a viral protein of a orthomyxovirus or variant thereof which induces a reaction of the immune system (i.e. immune response) in a host which is mediated by T cells, and the second polynucleotide encodes a viral protein of a orthomyxovirus or variant thereof that induces an anti-pathogenic B cell response against the orthomyxovirus. It is preferred that the orthomyxovirus whose viral proteins are encoded for by the first and second polynucleotide is selected from the genus of Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotoviris and Isavirus. In even more preferred embodiments, the orthomxyovirus is Influenzavirus A, preferably selected from the subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7. According to a preferred embodiment of the first aspect, the second polynucleotide encodes a viral protein of an

orthomyxovirus or variant thereof that induces an anti-pathogenic B cell response against the orthomyxovirus.

According to preferred embodiments of the first aspect first polynucleotide encodes a viral protein of a orthomyxovirus or variant thereof which induces a reaction of the immune system (i.e. immune response) in a host which is mediated by T cells. A T cell response involves the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells). A T cell response against a protein is induced, if peptides of the protein are processed within the cell and presented to T cells on the surface of the cell via the MHC I or MHC II pathway. Thus, in the context of the present invention preferably those viral proteins or parts thereof are used for inducing a T cell response that are normally not exposed on the outside of the virus, e.g. non structural or internal proteins or parts of structural or surface proteins not accessible to B-cells on the outside of the virus.

According to a preferred embodiment of the first aspect, the second polynucleotide encodes a viral protein of an orthomyxovirus or variant thereof that induces an anti-pathogenic B cell response. A B cell response is an immune response based on the activation of B lymphocytes, which produce and secrete antigen specific antibodies. B cells involved in such immune response include but are not limited to plasma B cells, memory B cells and B-1 cells. Thus, in the context of the present invention preferably those viral proteins or parts thereof are used for inducing a B cell response that are exposed on the outside of the virus, e.g. structural and/or surface proteins or at least those parts of structural and/or surface proteins accessible to B-cells on the outside of the virus.

In a preferred embodiment of the first aspect the viral protein of an orthomyxovirus, which induces a T cell response is a non-structural and/or internal protein of an orthomyxovirus, and/or the viral protein of a orthomyxovirus, which induces an anti-pathogenic B cell response is a structural and/or surface protein of a orthomyxovirus.

It is preferred that the amino acid sequence of the structural (surface) and/or non-structural and/or internal protein comprises consecutive segments or a consensus sequence of one or more different orthomyxovirus isolates.

In preferred embodiments, the structural protein is a protein exposed on the surface of the native orthomyxovirus or a variant thereof. It is preferred that the structural and/or surface protein triggers a T-cell independent immune response such as but not limited to an antibody mediated immune response or an activation of the complement system. In a particularly preferred embodiment, the structural and/or surface protein induces an antibody mediated immune response. Such antibody mediated immune response is based on the activation of B cells

which produce and secrete antigen specific antibodies. B cells involved in such immune response include but are not limited to plasma B cells, memory B cells and B-1 cells.

In a further preferred embodiment, the membrane attachment domain of the protein exposed on the surface of the native orthomyxovirus or variant thereof is functionally deleted, thus, either being structurally deleted or structurally present but not fulfilling its biological function. In a particularly preferred embodiment, the amino acid sequence corresponding to the membrane attachment domain is deleted. The deletion of the membrane attachment region serves the purpose of ascertaining that the anti-pathogenic B cell response inducing protein is secreted from the cell into which the expression system of the invention has been introduced.

It is further preferred that the viral surface proteins of the native orthomyxovirus is selected from the group consisting of hemagglutinin (HA) and neuraminidase (NA). It is more preferred that the viral surface protein of the native orthomyxovirus is hemagglutinin (HA).

In a preferred embodiment of the first aspect, HA comprises, essentially consists of or consists of an amino acid sequence of HA of one influenza A virus isolate or a consensus amino acid sequence of two or more different influenza A virus isolates, preferably according to SEQ ID NO: 8 or SEQ ID NO: 20, more preferably according to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 21 or a variant of one of these sequences.

In preferred embodiments of the first aspect, the non-structural protein is a conserved internal protein of orthomyxoviruses suitable for inducing a T cell mediated immune response against the paramyxovirus, involving the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells). Thus, preferably the T cell inducing protein of the orthomyxovirus does not comprise a secretion signal.

Preferably, the non-structural and/or internal protein is selected from the group consisting of nucleoprotein NP, Matrix proteins M1 and M2, non structural proteins NS1 and NS2/NEP, and the RNA polymerases PA, PB1, PB2 and the protein PB1-F2 (PB1F2).

The nucleoprotein NP is a structural protein which encapsidates the negative strand viral RNA. NP is one of the main determinants of species specificity.

The protein M1 is a matrix protein of the influenza virus. It forms a coat inside the viral envelope. The M1 protein binds to the viral RNA. It also has multiple regulatory functions, performed by interaction with the components of the host cell. The mechanisms regulated include a role in the export of the viral ribonucleoproteins from the host cell nucleus, inhibition of viral transcription, and a role in the virus assembly and budding. The M1 protein forms a layer under the patches of host cell membrane that are rich with the viral hemagglutinin, neuraminidase and M2 transmembrane proteins, and facilitates budding of the mature viruses.

The non-structural NS1 protein is created by the internal protein encoding, linear negative-sense, single stranded RNA, NS gene segment and which also codes for the nuclear export protein or NEP, formerly referred to as the NS2 protein, which mediates the export of vRNPs. NS1 also binds dsRNA. As a consequence of its binding to dsRNA, the NS1 protein blocks the activation of the dsRNA-activated protein kinase (PKR) in vitro. This kinase phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (elF-2 alpha), leading to a decrease in the rate of initiation of translation. In the absence of NS1, this pathway is inhibited during anti-viral response to halt all protein translation - thus stopping the synthesis of viral proteins; however, the influenza virus' NS1 protein is an agent that circumvents host defenses to allow viral gene transcription to occur.

In preferred embodiments, HA comprises an amino acid sequence of HA of one influenza A virus isolate or a consensus amino acid sequence of two or more different influenza A virus isolates, preferably according to SEQ ID NO: 9 or SEQ ID NO: 21, NP comprises an amino acid sequence of NP of one influenza A virus isolate or a consensus amino acid sequence of two or more different influenza A virus isolates, preferably according to SEQ ID NO: 11, and/or M1 comprises an amino acid sequence of M1 of one influenza A virus isolate or a consensus amino acid sequence of two or more different influenza A virus isolates, preferably according to SEQ ID NO: 12. It is further preferred that when NP comprises the amino acid sequence according to SEQ ID NO: 11 and M1 comprises the amino acid sequence according to SEQ ID NO: 12.

In the context of the present invention, the structural and/or surface protein encoded by the first polynucleotide is located either N- or C-terminally with respect to the non-structural and/or internal protein encoded by the second polynucleotide. In a preferred embodiment, the non-structural and/or internal protein encoded by the second polynucleotide is located C-terminally with respect to the structural and/or surface protein encoded by the first polynucleotide.

More specifically, HA or NA can be located N- or C-terminally of NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2). In a preferred embodiment M1 is located N-terminally of HA.

Accordingly, embodiments of the present invention have the formula X-Y or Y-X, wherein “X” depicts HA or NA, preferably HA, and “Y” depicts NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2), preferably NP or M1, and a “dash” depicts a peptide bond. Preferred arrangements are the following:

HA-NP, HA-M1, HA-M2, HA-NS1, HA-NS2/NEP, HA-PA, HA-PB1, HA-PB2, HA-PB1F2, NP-HA, M1-HA, M2-HA, NS1-HA, NS2/NEP-HA, PA-HA, PB1-HA, PB2-HA, PB1F2-HA, NA-NP, NA-M1, NA-M2, NA-NS1, NA-NS2/NEP, NA-PA, NA-PB1, NA-PB2,

NA-PB1F2, NP-NA, M1-NA, M2-NA, NS1-NA, NS2/NEP-NA, PA-NA, PB1-NA, PB2-NA or PB1F2-NA. A particulary preferred arrangement is M1-HA.

It is within the scope of the present invention that every protein can be combined with any other protein.

In preferred embodiments of the first aspect, a polynucleotide encoding a cleavage site is positioned between the first polynucleotide and the second polynucleotide.

It is preferred that this cleavage site is either a self-cleaving site (i.e. a cleavage site within the amino acid sequence where this sequence is cleaved or is cleavable without such cleavage involving any additional molecule or where the peptide-bond formation in this sequence is prevented in the first place) or an endopeptidase cleavage site (i.e. a cleavage cite within the amino acid sequence where this sequence is cleaved or is cleavable by an endopeptidase, e.g. trypsin, pepsin, elastase, thrombin, collagenase, furin, thermolysin, endopeptidase V8, cathepsins). More preferably, the self-cleaving site is a 2A cleavage site selected from the group consisting of a viral 2A peptide or 2A-like peptide of Picornavirus, insect viruses, Aphtoviridae, Rotaviruses and Trypanosoma, preferably wherein the 2A cleavage site is the 2 A peptide of foot and mouth disease virus. Alternatively or additionally, the polyprotein of the present invention can be cleaved by an autoprotease, i.e. a protease which cleaves peptide bonds in the same protein molecule which also comprises the protease. Examples of such autoproteases are the NS2 protease from flaviviruses or the VP4 protease of birnaviruses.

In the context of the present invention, the cleavage site can be positioned N-terminally with respect to the structural and/or surface protein encoded by the first polynucleotide and C-terminally with respect to the non-structural and/or internal protein encoded by the second polynucleotide. Alternatively the cleavage site can be positioned C-terminally with respect to the structural and/or surface protein encoded by the first polynucleotide and N-terminally with respect to the non-structural and/or internal protein encoded by the second polynucleotide. More specifically, the cleavage site can be positioned C- or N-terminally with respect to HA or NA and C- or N-terminally with respect to NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2). In a preferred embodiment the cleavage site is located C-terminally with respect to NP M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2) and N-terminally with respect to HA or NA. It is particularly preferred that the cleavage site is located N-terminally with respect to HA and C-terminally with respect to M1.

Accordingly, embodiments of the present invention have the formula X-C-Y or Y-C-X, wherein “X” depicts HA or NA, preferably, HA and “Y” depicts NP, M1, M2, NS1, NS2/NEP,

PA, PB1, PB2 or PB1-F2 (PB1F2), preferably NP or M1, "C" depicts a cleavage site, and a "dash" depicts a peptide bond.

Preferred arrangements are the following:

HA-C-NP, HA-C-M1, HA-C-M2, HA-C-NS1, HA-C-NS2/NEP, HA-C-PA, HA-C-PB1, HA-C-PB2, HA-C-PB1F2, NP-C-HA, M1-C-HA, M2-C-HA, NS1-C-HA, NS2/NEP-C-HA, PA-C-HA, PB1-C-HA, PB2-C-HA, PB1F2-C-HA, NA-C-NP, NA-C-M1, NA-C-M2, NA-C-NS1, NA-C-NS2/NEP, NA-C-PA, NA-C-PB1, NA-C-PB2, NA-C-PB1F2, NP-C-NA, M1-C-NA, M2-C-NA, NS1-C-NA, NS2/NEP-C-NA, PA-C-NA, PB1-C-NA, PB2-C-NA or PB1F2-C-NA. A particulary preferred arrangement is M1-C-HA.

It is within the scope of the present invention that every protein can be combined with any other protein and that any two proteins can or cannot be connected or linked by a cleavage site.

In preferred embodiment of the first aspect, the expression system further comprises a third polynucleotide encoding a non-structural and/or internal protein of an orthomyxovirus or a variant thereof. Preferably, the non-structural and/or internal protein is of a orthomyxovirus selected from the genus of Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotoviris and Isavirus. In even more preferred embodiments, the orthomxyovirus is Influenzavirus A, preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

In preferred embodiments the third polynucleotide is comprised on a separate or on the same vector as the first polynucleotide and/or the second polynucleotide.

Accordingly, the first polynucleotide is comprised on one vector and the second polynucleotide is comprised on a second vector and the third polynucleotide is comprised on a third vector. Alternatively or additionally, the first and the second polynucleotide are comprised on the same vector and the third polynucleotide is comprised on a separate vector, or the first and the third polynucleotide are comprised on the same vector and the second polynucleotide is comprised on a separate vector, or the second and the third polynucleotide are comprised on the same vector and the first polynucleotide is comprised on a separate vector. Alternatively or additionally, the first and the second and the third polynucleotide are comprised on the same vector. It is preferred that the first and the second and the third polynucleotide may be comprised on the same vector. It is particularly preferred that the first and the second and the third polynucleotide comprised on the same vector are linked in such that they are expressed as a viral polyprotein. Preferably, the first and the second and the third polynucleotide comprised on the same vector form an open reading frame.

It is further preferred that the non-structural and/or internal protein encoded by the third polynucleotide is a conserved internal protein suitable for inducing a T cell mediated immune response against the virus involving the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells).

Preferably, the non-structural and/or internal protein is selected from the group consisting of NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2), more preferably NP or M1.

It is preferred that the non-structural and/or internal protein encoded by the third polynucleotide differs from the non-structural and/or internal protein encoded by the second polynucleotide.

The non-structural and/or internal proteins encoded by the second and the third polynucleotide differ from each other in that they comprise amino acid sequences of different viral proteins. For instance, this means that the non-structural and/or internal protein encoded by the second polynucleotide comprises the amino acid sequence of the M1 protein whilst the non-structural and/or internal protein encoded by the third polynucleotide comprises the amino acid sequence of the NP protein or vice versa.

The non-structural and/or internal protein encoded by the third polynucleotide can be located either N- or C-terminally of the non-structural and/or internal protein encoded by the second polynucleotide. In a preferred embodiment of the first aspect, the non-structural and/or internal protein encoded by the third polynucleotide is located C-terminally of the non-structural and/or internal protein encoded by the second polynucleotide.

In preferred embodiments a polynucleotide encoding a linker is positioned between the second polynucleotide and the third polynucleotide. It is preferred that the linker is a flexible linker, preferably a flexible linker comprising an amino acid sequence according to SEQ ID NO: 6.

In embodiments of the first aspect, the protein encoded by the second polynucleotide is located N-terminally with respect to the protein encoded by the first polynucleotide and/or the protein of the optional third polynucleotide, or the protein encoded by the second polynucleotide is located C-terminally with respect to the protein encoded by the first polynucleotide and/or the protein of the optional third polynucleotide.

In even more preferred embodiments of this aspect, the first polynucleotide is located N-terminally with respect to the protein encoded by the second polynucleotide and/or the protein of the optional third polynucleotide is located N-terminally with respect to the protein encoded by the first polynucleotide or is located between the protein encoded by the second polynucleotide and the protein encoded by the first polynucleotide; or the protein encoded by the first

polynucleotide is located C-terminally with respect to the protein encoded by the second polynucleotide and/or the protein of the optional third polynucleotide is located C-terminally with respect to the protein encoded by the first polynucleotide or is located between the protein encoded by the second polynucleotide and the protein encoded by the first polynucleotide. More specifically, HA, or NA are located C- or N-terminally with respect to NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2).

In a preferred embodiment HA is located C-terminally with respect to M1 and NP is located N-terminally with respect to M1.

Accordingly, preferred embodiments of the present invention have the formula X-K-Y, Y-K-X, X-K-Y-Y, Y-Y-K-X, X-Y-K-Y, Y-K-Y-X, X-K-Y-K-Y, Y-K-Y-K-X, X-C-Y, Y-C-X, X-C-Y-Y, Y-Y-C-X, X-Y-C-Y, Y-C-Y-X, X-C-Y-C-Y, Y-C-Y-C-X, X-K-Y-C-Y, Y-C-Y-K-X, X-C-Y-K-Y, or Y-K-Y-C-X, wherein "X" depicts HA, or NA, preferably HA, and "Y" depicts NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2), preferably NP or M1, "K" indicates that one or more peptide linkers are present in this position, "C" indicates that one or more cleavage sites are present in this position and a "dash" depicts a peptide bond. Preferred arrangements are Y-K-Y-C-X. Even more preferred arrangements are the following:
HA-K-NP, NP-K-HA, HA-K-NP-NP, NP-NP-K-HA, HA-NP-K-NP, NP-K-NP-HA, HA-K-NP-K-NP, NP-K-NP-K-HA, HA-C-NP, NP-C-HA, HA-C-NP-NP, NP-NP-C-HA, HA-NP-C-NP, NP-C-NP-HA, HA-C-NP-C-NP, NP-C-NP-C-HA, HA-K-NP-C-NP, NP-C-NP-K-HA, HA-C-NP-K-NP, NP-K-NP-C-HA, HA-K-NP-M1, HA-K-M1-NP, NP-M1-K-HA, M1-NP-K-HA, HA-NP-K-M1, HA-M1-K-NP, NP-K-M1-HA, M1-K-NP-HA, HA-K-NP-K-M1, HA-K-M1-K-NP, NP-K-M1-K-HA, M1-K-NP-K-HA, HA-C-NP-M1, HA-C-M1-NP, NP-M1-C-HA, M1-NP-C-HA, HA-NP-C-M1, HA-M1-C-NP, NP-C-M1-HA, M1-C-NP-HA, HA-C-NP-C-M1, HA-C-M1-C-NP, NP-C-M1-C-HA, M1-C-NP-C-HA, HA-K-NP-C-M1, HA-K-M1-C-NP, NP-C-M1-K-HA, M1-C-NP-K-HA, HA-C-NP-K-M1, HA-C-M1-K-NP, NP-K-M1-C-HA, M1-K-NP-C-HA, HA-K-NP-M2, HA-K-M2-NP, NP-M2-K-HA, M2-NP-K-HA, HA-NP-K-M2, HA-M2-K-NP, NP-K-M2-HA, M2-K-NP-HA, HA-K-NP-K-M2, HA-K-M2-K-NP, NP-K-M2-K-HA, M2-K-NP-K-HA, HA-C-NP-M2, HA-C-M2-NP, NP-M2-C-HA, M2-NP-C-HA, HA-NP-C-M2, HA-M2-C-NP, NP-C-M2-HA, M2-C-NP-HA, HA-C-NP-C-M2, HA-C-M2-C-NP, NP-C-M2-C-HA, M2-C-NP-C-HA, HA-K-NP-C-M2, HA-K-M2-C-NP, NP-C-M2-K-HA, M2-C-NP-K-HA, HA-C-NP-K-M2, HA-C-M2-K-NP, NP-K-M2-C-HA, M2-K-NP-C-HA, HA-K-NP-NS1, HA-K-NS1-NP, NP-NS1-K-HA, NS1-NP-K-HA, HA-NP-K-NS1, HA-NS1-K-NP, NP-K-NS1-HA, NS1-K-NP-HA, HA-K-NP-K-NS1, HA-K-NS1-K-NP, NP-K-NS1-K-HA, NS1-K-NP-K-HA, HA-C-NP-NS1, HA-C-NS1-NP, NP-NS1-C-HA, NS1-NP-C-HA, HA-NP-C-NS1, HA-NS1-C-NP, NP-C-NS1-HA, NS1-C-NP-HA, HA-C-NP-C-NS1, HA-C-NS1-C-NP, NP-C-NS1-C-HA,

NS1-C-NP-C-HA, HA-K-NP-C-NS1, HA-K-NS1-C-NP, NP-C-NS1-K-HA, NS1-C-NP-K-HA, HA-C-NP-K-NS1, HA-C-NS1-K-NP, NP-K-NS1-C-HA, NS1-K-NP-C-HA, HA-K-NP-NS2/NEP, HA-K-NS2/NEP-NP, NP-NS2/NEP-K-HA, NS2/NEP-NP-K-HA, HA-NP-K-NS2/NEP, HA-NS2/NEP-K-NP, NP-K-NS2/NEP-HA, NS2/NEP-K-NP-HA, HA-K-NP-K-NS2/NEP, HA-K-NS2/NEP-K-NP, NP-K-NS2/NEP-K-HA, NS2/NEP-K-NP-K-HA, HA-C-NP-NS2/NEP, HA-C-NS2/NEP-NP, NP-NS2/NEP-C-HA, NS2/NEP-NP-C-HA, HA-NP-C-NS2/NEP, HA-NS2/NEP-C-NP, NP-C-NS2/NEP-HA, NS2/NEP-C-NP-HA, HA-C-NP-C-NS2/NEP, HA-C-NS2/NEP-C-NP, NP-C-NS2/NEP-C-HA, NS2/NEP-C-NP-C-HA, HA-K-NP-C-NS2/NEP, HA-K-NS2/NEP-C-NP, NP-C-NS2/NEP-K-HA, NS2/NEP-C-NP-K-HA, HA-C-NP-K-NS2/NEP, HA-C-NS2/NEP-K-NP, NP-K-NS2/NEP-C-HA, NS2/NEP-K-NP-C-HA, HA-K-NP-PA, HA-K-PA-NP, NP-PA-K-HA, PA-NP-K-HA, HA-NP-K-PA, HA-PA-K-NP, NP-K-PA-HA, PA-K-NP-HA, HA-K-NP-K-PA, HA-K-PA-K-NP, NP-K-PA-K-HA, PA-K-NP-K-HA, HA-C-NP-PA, HA-C-PA-NP, NP-PA-C-HA, PA-NP-C-HA, HA-NP-C-PA, HA-PA-C-NP, NP-C-PA-HA, PA-C-NP-HA, HA-C-NP-C-PA, HA-C-PA-C-NP, NP-C-PA-C-HA, PA-C-NP-C-HA, HA-K-NP-C-PA, HA-K-PA-C-NP, NP-C-PA-K-HA, PA-C-NP-K-HA, HA-C-NP-K-PA, HA-C-PA-K-NP, NP-K-PA-C-HA, PA-K-NP-C-HA, HA-K-NP-PB1, HA-K-PB1-NP, NP-PB1-K-HA, PB1-NP-K-HA, HA-NP-K-PB1, HA-PB1-K-NP, NP-K-PB1-HA, PB1-K-NP-HA, HA-K-NP-K-PB1, HA-K-PB1-K-NP, NP-K-PB1-K-HA, PB1-K-NP-K-HA, HA-C-NP-PB1, HA-C-PB1-NP, NP-PB1-C-HA, PB1-NP-C-HA, HA-NP-C-PB1, HA-PB1-C-NP, NP-C-PB1-HA, PB1-C-NP-HA, HA-C-NP-C-PB1, HA-C-PB1-C-NP, NP-C-PB1-C-HA, PB1-C-NP-C-HA, HA-K-NP-C-PB1, HA-K-PB1-C-NP, NP-C-PB1-K-HA, PB1-C-NP-K-HA, HA-C-NP-K-PB1, HA-C-PB1-K-NP, NP-K-PB1-C-HA, PB1-K-NP-C-HA, HA-K-NP-PB2, HA-K-PB2-NP, NP-PB2-K-HA, PB2-NP-K-HA, HA-NP-K-PB2, HA-PB2-K-NP, NP-K-PB2-HA, PB2-K-NP-HA, HA-K-NP-K-PB2, HA-K-PB2-K-NP, NP-K-PB2-K-HA, PB2-K-NP-K-HA, HA-C-NP-PB2, HA-C-PB2-NP, NP-PB2-C-HA, PB2-NP-C-HA, HA-NP-C-PB2, HA-PB2-C-NP, NP-C-PB2-HA, PB2-C-NP-HA, HA-C-NP-C-PB2, HA-C-PB2-C-NP, NP-C-PB2-C-HA, PB2-C-NP-C-HA, HA-K-NP-C-PB2, HA-K-PB2-C-NP, NP-C-PB2-K-HA, PB2-C-NP-K-HA, HA-C-NP-K-PB2, HA-C-PB2-K-NP, NP-K-PB2-C-HA, PB2-K-NP-C-HA, HA-K-NP-PB1F2, HA-K-PB1F2-NP, NP-PB1F2-K-HA, PB1F2-NP-K-HA, HA-NP-K-PB1F2, HA-PB1F2-K-NP, NP-K-PB1F2-HA, PB1F2-K-NP-HA, HA-K-NP-K-PB1F2, HA-K-PB1F2-K-NP, NP-K-PB1F2-K-HA, PB1F2-K-NP-K-HA, HA-C-NP-PB1F2, HA-C-PB1F2-NP, NP-PB1F2-C-HA, PB1F2-NP-C-HA, HA-NP-C-PB1F2, HA-PB1F2-C-NP, NP-C-PB1F2-HA, PB1F2-C-NP-HA, HA-C-NP-C-PB1F2, HA-C-PB1F2-C-NP, NP-C-PB1F2-C-HA, PB1F2-C-NP-C-HA, HA-K-NP-C-PB1F2, HA-K-PB1F2-C-NP, NP-C-PB1F2-K-HA, PB1F2-C-NP-K-HA, HA-C-NP-K-PB1F2, HA-C-PB1F2-K-NP, NP-K-PB1F2-C-HA, PB1F2-K-NP-C-HA, HA-K-M1, M1-K-HA, HA-K-M1-M1, M1-M1-K-HA,

HA-M1-K-M1, M1-K-M1-HA, HA-K-M1-K-M1, M1-K-M1-K-HA, HA-C-M1, M1-C-HA, HA-C-M1-M1, M1-M1-C-HA, HA-M1-C-M1, M1-C-M1-HA, HA-C-M1-C-M1, M1-C-M1-C-HA, HA-K-M1-C-M1, M1-C-M1-K-HA, HA-C-M1-K-M1, M1-K-M1-C-HA, HA-K-M1-M2, HA-K-M2-M1, M1-M2-K-HA, M2-M1-K-HA, HA-M1-K-M2, HA-M2-K-M1, M1-K-M2-HA, M2-K-M1-HA, HA-K-M1-K-M2, HA-K-M2-K-M1, M1-K-M2-K-HA, M2-K-M1-K-HA, HA-C-M1-M2, HA-C-M2-M1, M1-M2-C-HA, M2-M1-C-HA, HA-M1-C-M2, HA-M2-C-M1, M1-C-M2-HA, M2-C-M1-HA, HA-C-M1-C-M2, HA-C-M2-C-M1, M1-C-M2-C-HA, M2-C-M1-C-HA, HA-K-M1-C-M2, HA-K-M2-C-M1, M1-C-M2-K-HA, M2-C-M1-K-HA, HA-C-M1-K-M2, HA-C-M2-K-M1, M1-K-M2-C-HA, M2-K-M1-C-HA, HA-K-M1-NS1, HA-K-NS1-M1, M1-NS1-K-HA, NS1-M1-K-HA, HA-M1-K-NS1, HA-NS1-K-M1, M1-K-NS1-HA, NS1-K-M1-HA, HA-K-M1-K-NS1, HA-K-NS1-K-M1, M1-K-NS1-K-HA, NS1-K-M1-K-HA, HA-C-M1-NS1, HA-C-NS1-M1, M1-NS1-C-HA, NS1-M1-C-HA, HA-M1-C-NS1, HA-NS1-C-M1, M1-C-NS1-HA, NS1-C-M1-HA, HA-C-M1-C-NS1, HA-C-NS1-C-M1, M1-C-NS1-C-HA, NS1-C-M1-C-HA, HA-K-M1-C-NS1, HA-K-NS1-C-M1, M1-C-NS1-K-HA, NS1-C-M1-K-HA, HA-C-M1-K-NS1, HA-C-NS1-K-M1, M1-K-NS1-C-HA, NS1-K-M1-C-HA, HA-K-M1-NS2/NEP, HA-K-NS2/NEP-M1, M1-NS2/NEP-K-HA, NS2/NEP-M1-K-HA, HA-M1-K-NS2/NEP, HA-NS2/NEP-K-M1, M1-K-NS2/NEP-HA, NS2/NEP-K-M1-HA, HA-K-M1-K-NS2/NEP, HA-K-NS2/NEP-K-M1, M1-K-NS2/NEP-K-HA, NS2/NEP-K-M1-K-HA, HA-C-M1-NS2/NEP, HA-C-NS2/NEP-M1, M1-NS2/NEP-C-HA, NS2/NEP-M1-C-HA, HA-M1-C-NS2/NEP, HA-NS2/NEP-C-M1, M1-C-NS2/NEP-HA, NS2/NEP-C-M1-HA, HA-C-M1-C-NS2/NEP, HA-C-NS2/NEP-C-M1, M1-C-NS2/NEP-C-HA, NS2/NEP-C-M1-C-HA, HA-K-M1-C-NS2/NEP, HA-K-NS2/NEP-C-M1, M1-C-NS2/NEP-K-HA, NS2/NEP-C-M1-K-HA, HA-C-M1-K-NS2/NEP, HA-C-NS2/NEP-K-M1, M1-K-NS2/NEP-C-HA, NS2/NEP-K-M1-C-HA, HA-K-M1-PA, HA-K-PA-M1, M1-PA-K-HA, PA-M1-K-HA, HA-M1-K-PA, HA-PA-K-M1, M1-K-PA-HA, PA-K-M1-HA, HA-K-M1-K-PA, HA-K-PA-K-M1, M1-K-PA-K-HA, PA-K-M1-K-HA, HA-C-M1-PA, HA-C-PA-M1, M1-PA-C-HA, PA-M1-C-HA, HA-M1-C-PA, HA-PA-C-M1, M1-C-PA-HA, PA-C-M1-HA, HA-C-M1-C-PA, HA-C-PA-C-M1, M1-C-PA-C-HA, PA-C-M1-C-HA, HA-K-M1-C-PA, HA-K-PA-C-M1, M1-C-PA-K-HA, PA-C-M1-K-HA, HA-C-M1-K-PA, HA-C-PA-K-M1, M1-K-PA-C-HA, PA-K-M1-C-HA, HA-K-M1-PB1, HA-K-PB1-M1, M1-PB1-K-HA, PB1-M1-K-HA, HA-M1-K-PB1, HA-PB1-K-M1, M1-K-PB1-HA, PB1-K-M1-HA, HA-K-M1-K-PB1, HA-K-PB1-K-M1, M1-K-PB1-K-HA, PB1-K-M1-K-HA, HA-C-M1-PB1, HA-C-PB1-M1, M1-PB1-C-HA, PB1-M1-C-HA, HA-M1-C-PB1, HA-PB1-C-M1, M1-C-PB1-HA, PB1-C-M1-HA, HA-C-M1-C-PB1, HA-C-PB1-C-M1, M1-C-PB1-C-HA, PB1-C-M1-C-HA, HA-K-M1-C-PB1, HA-K-PB1-C-M1, M1-C-PB1-K-HA, PB1-C-M1-K-HA, HA-C-M1-K-PB1, HA-C-PB1-K-M1, M1-K-PB1-C-HA, PB1-K-M1-C-HA, HA-K-M1-PB2, HA-K-PB2-M1, M1-

PB2-K-HA, PB2-M1-K-HA, HA-M1-K-PB2, HA-PB2-K-M1, M1-K-PB2-HA, PB2-K-M1-HA, HA-K-M1-K-PB2, HA-K-PB2-K-M1, M1-K-PB2-K-HA, PB2-K-M1-K-HA, HA-C-M1-PB2, HA-C-PB2-M1, M1-PB2-C-HA, PB2-M1-C-HA, HA-M1-C-PB2, HA-PB2-C-M1, M1-C-PB2-HA, PB2-C-M1-HA, HA-C-M1-C-PB2, HA-C-PB2-C-M1, M1-C-PB2-C-HA, PB2-C-M1-C-HA, HA-K-M1-C-PB2, HA-K-PB2-C-M1, M1-C-PB2-K-HA, PB2-C-M1-K-HA, HA-C-M1-K-PB2, HA-C-PB2-K-M1, M1-K-PB2-C-HA, PB2-K-M1-C-HA, HA-K-M1-PB1F2, HA-K-PB1F2-M1, M1-PB1F2-K-HA, PB1F2-M1-K-HA, HA-M1-K-PB1F2, HA-PB1F2-K-M1, M1-K-PB1F2-HA, PB1F2-K-M1-HA, HA-K-M1-K-PB1F2, HA-K-PB1F2-K-M1, M1-K-PB1F2-K-HA, PB1F2-K-M1-K-HA, HA-C-M1-PB1F2, HA-C-PB1F2-M1, M1-PB1F2-C-HA, PB1F2-M1-C-HA, HA-M1-C-PB1F2, HA-PB1F2-C-M1, M1-C-PB1F2-HA, PB1F2-C-M1-HA, HA-C-M1-C-PB1F2, HA-C-PB1F2-C-M1, M1-C-PB1F2-C-HA, PB1F2-C-M1-C-HA, HA-K-M1-C-PB1F2, HA-K-PB1F2-C-M1, M1-C-PB1F2-K-HA, PB1F2-C-M1-K-HA, HA-C-M1-K-PB1F2, HA-C-PB1F2-K-M1, M1-K-PB1F2-C-HA, PB1F2-K-M1-C-HA, HA-K-M2, M2-K-HA, HA-K-M2-M2, M2-M2-K-HA, HA-M2-K-M2, M2-K-M2-HA, HA-K-M2-K-M2, M2-K-M2-K-HA, HA-C-M2, M2-C-HA, HA-C-M2-M2, M2-M2-C-HA, HA-M2-C-M2, M2-C-M2-HA, HA-C-M2-C-M2, M2-C-M2-C-HA, HA-K-M2-C-M2, M2-C-M2-K-HA, HA-C-M2-K-M2, M2-K-M2-C-HA, HA-K-M2-NS1, HA-K-NS1-M2, M2-NS1-K-HA, NS1-M2-K-HA, HA-M2-K-NS1, HA-NS1-K-M2, M2-K-NS1-HA, NS1-K-M2-HA, HA-K-M2-K-NS1, HA-K-NS1-K-M2, M2-K-NS1-K-HA, NS1-K-M2-K-HA, HA-C-M2-NS1, HA-C-NS1-M2, M2-NS1-C-HA, NS1-M2-C-HA, HA-M2-C-NS1, HA-NS1-C-M2, M2-C-NS1-HA, NS1-C-M2-HA, HA-C-M2-C-NS1, HA-C-NS1-C-M2, M2-C-NS1-C-HA, NS1-C-M2-C-HA, HA-K-M2-C-NS1, HA-K-NS1-C-M2, M2-C-NS1-K-HA, NS1-C-M2-K-HA, HA-C-M2-K-NS1, HA-C-NS1-K-M2, M2-K-NS1-C-HA, NS1-K-M2-C-HA, HA-K-M2-NS2/NEP, HA-K-NS2/NEP-M2, M2-NS2/NEP-K-HA, NS2/NEP-M2-K-HA, HA-M2-K-NS2/NEP, HA-NS2/NEP-K-M2, M2-K-NS2/NEP-HA, NS2/NEP-K-M2-HA, HA-K-M2-K-NS2/NEP, HA-K-NS2/NEP-K-M2, M2-K-NS2/NEP-K-HA, NS2/NEP-K-M2-K-HA, HA-C-M2-NS2/NEP, HA-C-NS2/NEP-M2, M2-NS2/NEP-C-HA, NS2/NEP-M2-C-HA, HA-M2-C-NS2/NEP, HA-NS2/NEP-C-M2, M2-C-NS2/NEP-HA, NS2/NEP-C-M2-HA, HA-C-M2-C-NS2/NEP, HA-C-NS2/NEP-C-M2, M2-C-NS2/NEP-C-HA, NS2/NEP-C-M2-C-HA, HA-K-M2-C-NS2/NEP, HA-K-NS2/NEP-C-M2, M2-C-NS2/NEP-K-HA, NS2/NEP-C-M2-K-HA, HA-C-M2-K-NS2/NEP, HA-C-NS2/NEP-K-M2, M2-K-NS2/NEP-C-HA, NS2/NEP-K-M2-C-HA, HA-K-M2-PA, HA-K-PA-M2, M2-PA-K-HA, PA-M2-K-HA, HA-M2-K-PA, HA-PA-K-M2, M2-K-PA-HA, PA-K-M2-HA, HA-K-M2-K-PA, HA-K-PA-K-M2, M2-K-PA-K-HA, PA-K-M2-K-HA, HA-C-M2-PA, HA-C-PA-M2, M2-PA-C-HA, PA-M2-C-HA, HA-M2-C-PA, HA-PA-C-M2, M2-C-PA-HA, PA-C-M2-HA, HA-C-M2-C-PA, HA-C-PA-C-M2, M2-C-PA-C-HA, PA-C-M2-C-HA, HA-K-M2-C-PA, HA-K-PA-C-

M2, M2-C-PA-K-HA, PA-C-M2-K-HA, HA-C-M2-K-PA, HA-C-PA-K-M2, M2-K-PA-C-HA, PA-K-M2-C-HA, HA-K-M2-PB1, HA-K-PB1-M2, M2-PB1-K-HA, PB1-M2-K-HA, HA-M2-K-PB1, HA-PB1-K-M2, M2-K-PB1-HA, PB1-K-M2-HA, HA-K-M2-K-PB1, HA-K-PB1-K-M2, M2-K-PB1-K-HA, PB1-K-M2-K-HA, HA-C-M2-PB1, HA-C-PB1-M2, M2-PB1-C-HA, PB1-M2-C-HA, HA-M2-C-PB1, HA-PB1-C-M2, M2-C-PB1-HA, PB1-C-M2-HA, HA-C-M2-C-PB1, HA-C-PB1-C-M2, M2-C-PB1-C-HA, PB1-C-M2-C-HA, HA-K-M2-C-PB1, HA-K-PB1-C-M2, M2-C-PB1-K-HA, PB1-C-M2-K-HA, HA-C-M2-K-PB1, HA-C-PB1-K-M2, M2-K-PB1-C-HA, PB1-K-M2-C-HA, HA-K-M2-PB2, HA-K-PB2-M2, M2-PB2-K-HA, PB2-M2-K-HA, HA-M2-K-PB2, HA-PB2-K-M2, M2-K-PB2-HA, PB2-K-M2-HA, HA-K-M2-K-PB2, HA-K-PB2-K-M2, M2-K-PB2-K-HA, PB2-K-M2-K-HA, HA-C-M2-PB2, HA-C-PB2-M2, M2-PB2-C-HA, PB2-M2-C-HA, HA-M2-C-PB2, HA-PB2-C-M2, M2-C-PB2-HA, PB2-C-M2-HA, HA-C-M2-C-PB2, HA-C-PB2-C-M2, M2-C-PB2-C-HA, PB2-C-M2-C-HA, HA-K-M2-C-PB2, HA-K-PB2-C-M2, M2-C-PB2-K-HA, PB2-C-M2-K-HA, HA-C-M2-K-PB2, HA-C-PB2-K-M2, M2-K-PB2-C-HA, PB2-K-M2-C-HA, HA-K-M2-PB1F2, HA-K-PB1F2-M2, M2-PB1F2-K-HA, PB1F2-M2-K-HA, HA-M2-K-PB1F2, HA-PB1F2-K-M2, M2-K-PB1F2-HA, PB1F2-K-M2-HA, HA-K-M2-K-PB1F2, HA-K-PB1F2-K-M2, M2-K-PB1F2-K-HA, PB1F2-K-M2-K-HA, HA-C-M2-PB1F2, HA-C-PB1F2-M2, M2-PB1F2-C-HA, PB1F2-M2-C-HA, HA-M2-C-PB1F2, HA-PB1F2-C-M2, M2-C-PB1F2-HA, PB1F2-C-M2-HA, HA-C-M2-C-PB1F2, HA-C-PB1F2-C-M2, M2-C-PB1F2-C-HA, PB1F2-C-M2-C-HA, HA-K-M2-C-PB1F2, HA-K-PB1F2-C-M2, M2-C-PB1F2-K-HA, PB1F2-C-M2-K-HA, HA-C-M2-K-PB1F2, HA-C-PB1F2-K-M2, M2-K-PB1F2-C-HA, PB1F2-K-M2-C-HA, HA-K-NS1, NS1-K-HA, HA-K-NS1-NS1, NS1-NS1-K-HA, HA-NS1-K-NS1, NS1-K-NS1-HA, HA-K-NS1-K-NS1, NS1-K-NS1-K-HA, HA-C-NS1, NS1-C-HA, HA-C-NS1-NS1, NS1-NS1-C-HA, HA-NS1-C-NS1, NS1-C-NS1-HA, HA-C-NS1-C-NS1, NS1-C-NS1-C-HA, HA-K-NS1-C-NS1, NS1-C-NS1-K-HA, HA-C-NS1-K-NS1, NS1-K-NS1-C-HA, HA-K-NS1-NS2/NEP, HA-K-NS2/NEP-NS1, NS1-NS2/NEP-K-HA, NS2/NEP-NS1-K-HA, HA-NS1-K-NS2/NEP, HA-NS2/NEP-K-NS1, NS1-K-NS2/NEP-HA, NS2/NEP-K-NS1-HA, HA-K-NS1-K-NS2/NEP, HA-K-NS2/NEP-K-NS1, NS1-K-NS2/NEP-K-HA, NS2/NEP-K-NS1-K-HA, HA-C-NS1-NS2/NEP, HA-C-NS2/NEP-NS1, NS1-NS2/NEP-C-HA, NS2/NEP-NS1-C-HA, HA-NS1-C-NS2/NEP, HA-NS2/NEP-C-NS1, NS1-C-NS2/NEP-HA, NS2/NEP-C-NS1-HA, HA-C-NS1-C-NS2/NEP, HA-C-NS2/NEP-C-NS1, NS1-C-NS2/NEP-C-HA, NS2/NEP-C-NS1-C-HA, HA-K-NS1-C-NS2/NEP, HA-K-NS2/NEP-C-NS1, NS1-C-NS2/NEP-K-HA, NS2/NEP-C-NS1-K-HA, HA-C-NS1-K-NS2/NEP, HA-C-NS2/NEP-K-NS1, NS1-K-NS2/NEP-C-HA, NS2/NEP-K-NS1-C-HA, HA-K-NS1-PA, HA-K-PA-NS1, NS1-PA-K-HA, PA-NS1-K-HA, HA-NS1-K-PA, HA-PA-K-NS1, NS1-K-PA-HA, PA-K-NS1-HA, HA-K-NS1-K-PA, HA-K-PA-K-NS1, NS1-K-PA-K-HA, PA-K-NS1-K-HA, HA-C-NS1-PA, HA-C-PA-

NS1, NS1-PA-C-HA, PA-NS1-C-HA, HA-NS1-C-PA, HA-PA-C-NS1, NS1-C-PA-HA, PA-C-NS1-HA, HA-C-NS1-C-PA, HA-C-PA-C-NS1, NS1-C-PA-C-HA, PA-C-NS1-C-HA, HA-K-NS1-C-PA, HA-K-PA-C-NS1, NS1-C-PA-K-HA, PA-C-NS1-K-HA, HA-C-NS1-K-PA, HA-C-PA-K-NS1, NS1-K-PA-C-HA, PA-K-NS1-C-HA, HA-K-NS1-PB1, HA-K-PB1-NS1, NS1-PB1-K-HA, PB1-NS1-K-HA, HA-NS1-K-PB1, HA-PB1-K-NS1, NS1-K-PB1-HA, PB1-K-NS1-HA, HA-K-NS1-K-PB1, HA-K-PB1-K-NS1, NS1-K-PB1-K-HA, PB1-K-NS1-K-HA, HA-C-NS1-PB1, HA-C-PB1-NS1, NS1-PB1-C-HA, PB1-NS1-C-HA, HA-NS1-C-PB1, HA-PB1-C-NS1, NS1-C-PB1-HA, PB1-C-NS1-HA, HA-C-NS1-C-PB1, HA-C-PB1-C-NS1, NS1-C-PB1-C-HA, PB1-C-NS1-C-HA, HA-K-NS1-C-PB1, HA-K-PB1-C-NS1, NS1-C-PB1-K-HA, PB1-C-NS1-K-HA, HA-C-NS1-K-PB1, HA-C-PB1-K-NS1, NS1-K-PB1-C-HA, PB1-K-NS1-C-HA, HA-K-NS1-PB2, HA-K-PB2-NS1, NS1-PB2-K-HA, PB2-NS1-K-HA, HA-NS1-K-PB2, HA-PB2-K-NS1, NS1-K-PB2-HA, PB2-K-NS1-HA, HA-K-NS1-K-PB2, HA-K-PB2-K-NS1, NS1-K-PB2-K-HA, PB2-K-NS1-K-HA, HA-C-NS1-PB2, HA-C-PB2-NS1, NS1-PB2-C-HA, PB2-NS1-C-HA, HA-NS1-C-PB2, HA-PB2-C-NS1, NS1-C-PB2-HA, PB2-C-NS1-HA, HA-C-NS1-C-PB2, HA-C-PB2-C-NS1, NS1-C-PB2-C-HA, PB2-C-NS1-C-HA, HA-K-NS1-C-PB2, HA-K-PB2-C-NS1, NS1-C-PB2-K-HA, PB2-C-NS1-K-HA, HA-C-NS1-K-PB2, HA-C-PB2-K-NS1, NS1-K-PB2-C-HA, PB2-K-NS1-C-HA, HA-K-NS1-PB1F2, HA-K-PB1F2-NS1, NS1-PB1F2-K-HA, PB1F2-NS1-K-HA, HA-NS1-K-PB1F2, HA-PB1F2-K-NS1, NS1-K-PB1F2-HA, PB1F2-K-NS1-HA, HA-K-NS1-K-PB1F2, HA-K-PB1F2-K-NS1, NS1-K-PB1F2-K-HA, PB1F2-K-NS1-K-HA, HA-C-NS1-PB1F2, HA-C-PB1F2-NS1, NS1-PB1F2-C-HA, PB1F2-NS1-C-HA, HA-NS1-C-PB1F2, HA-PB1F2-C-NS1, NS1-C-PB1F2-HA, PB1F2-C-NS1-HA, HA-C-NS1-C-PB1F2, HA-C-PB1F2-C-NS1, NS1-C-PB1F2-C-HA, PB1F2-C-NS1-C-HA, HA-K-NS1-C-PB1F2, HA-K-PB1F2-C-NS1, NS1-C-PB1F2-K-HA, PB1F2-C-NS1-K-HA, HA-C-NS1-K-PB1F2, HA-C-PB1F2-K-NS1, NS1-K-PB1F2-C-HA, PB1F2-K-NS1-C-HA, HA-K-NS2/NEP, NS2/NEP-K-HA, HA-K-NS2/NEP-NS2/NEP, NS2/NEP-NS2/NEP-K-HA, HA-NS2/NEP-K-NS2/NEP, NS2/NEP-K-NS2/NEP-HA, HA-K-NS2/NEP-K-NS2/NEP, NS2/NEP-K-NS2/NEP-K-HA, HA-C-NS2/NEP, NS2/NEP-C-HA, HA-C-NS2/NEP-NS2/NEP, NS2/NEP-NS2/NEP-C-HA, HA-NS2/NEP-C-NS2/NEP, NS2/NEP-C-NS2/NEP-HA, HA-C-NS2/NEP-C-NS2/NEP, NS2/NEP-C-NS2/NEP-C-HA, HA-K-NS2/NEP-C-NS2/NEP, NS2/NEP-C-NS2/NEP-K-HA, HA-C-NS2/NEP-K-NS2/NEP, NS2/NEP-K-NS2/NEP-C-HA, HA-K-NS2/NEP-PA, HA-K-PA-NS2/NEP, NS2/NEP-PA-K-HA, PA-NS2/NEP-K-HA, HA-NS2/NEP-K-PA, HA-PA-K-NS2/NEP, NS2/NEP-K-PA-HA, PA-K-NS2/NEP-HA, HA-K-NS2/NEP-K-PA, HA-K-PA-K-NS2/NEP, NS2/NEP-K-PA-K-HA, PA-K-NS2/NEP-K-HA, HA-C-NS2/NEP-PA, HA-C-PA-NS2/NEP, NS2/NEP-PA-C-HA, PA-NS2/NEP-C-HA, HA-NS2/NEP-C-PA, HA-PA-C-NS2/NEP, NS2/NEP-C-PA-HA, PA-C-NS2/NEP-HA, HA-C-

NS2/NEP-C-PA, HA-C-PA-C-NS2/NEP, NS2/NEP-C-PA-C-HA, PA-C-NS2/NEP-C-HA, HA-K-NS2/NEP-C-PA, HA-K-PA-C-NS2/NEP, NS2/NEP-C-PA-K-HA, PA-C-NS2/NEP-K-HA, HA-C-NS2/NEP-K-PA, HA-C-PA-K-NS2/NEP, NS2/NEP-K-PA-C-HA, PA-K-NS2/NEP-C-HA, HA-K-NS2/NEP-PB1, HA-K-PB1-NS2/NEP, NS2/NEP-PB1-K-HA, PB1-NS2/NEP-K-HA, HA-NS2/NEP-K-PB1, HA-PB1-K-NS2/NEP, NS2/NEP-K-PB1-HA, PB1-K-NS2/NEP-HA, HA-K-NS2/NEP-K-PB1, HA-K-PB1-K-NS2/NEP, NS2/NEP-K-PB1-K-HA, PB1-K-NS2/NEP-K-HA, HA-C-NS2/NEP-PB1, HA-C-PB1-NS2/NEP, NS2/NEP-PB1-C-HA, PB1-NS2/NEP-C-HA, HA-NS2/NEP-C-PB1, HA-PB1-C-NS2/NEP, NS2/NEP-C-PB1-HA, PB1-C-NS2/NEP-HA, HA-C-NS2/NEP-C-PB1, HA-C-PB1-C-NS2/NEP, NS2/NEP-C-PB1-C-HA, PB1-C-NS2/NEP-C-HA, HA-K-NS2/NEP-C-PB1, HA-K-PB1-C-NS2/NEP, NS2/NEP-C-PB1-K-HA, PB1-C-NS2/NEP-K-HA, HA-C-NS2/NEP-K-PB1, HA-C-PB1-K-NS2/NEP, NS2/NEP-K-PB1-C-HA, PB1-K-NS2/NEP-C-HA, HA-K-NS2/NEP-PB2, HA-K-PB2-NS2/NEP, NS2/NEP-PB2-K-HA, PB2-NS2/NEP-K-HA, HA-NS2/NEP-K-PB2, HA-PB2-K-NS2/NEP, NS2/NEP-K-PB2-HA, PB2-K-NS2/NEP-HA, HA-K-NS2/NEP-K-PB2, HA-K-PB2-K-NS2/NEP, NS2/NEP-K-PB2-K-HA, PB2-K-NS2/NEP-K-HA, HA-C-NS2/NEP-PB2, HA-C-PB2-NS2/NEP, NS2/NEP-PB2-C-HA, PB2-NS2/NEP-C-HA, HA-NS2/NEP-C-PB2, HA-PB2-C-NS2/NEP, NS2/NEP-C-PB2-HA, PB2-C-NS2/NEP-HA, HA-C-NS2/NEP-C-PB2, HA-C-PB2-C-NS2/NEP, NS2/NEP-C-PB2-C-HA, PB2-C-NS2/NEP-C-HA, HA-K-NS2/NEP-C-PB2, HA-K-PB2-C-NS2/NEP, NS2/NEP-C-PB2-K-HA, PB2-C-NS2/NEP-K-HA, HA-C-NS2/NEP-K-PB2, HA-C-PB2-K-NS2/NEP, NS2/NEP-K-PB2-C-HA, PB2-K-NS2/NEP-C-HA, HA-K-NS2/NEP-PB1F2, HA-K-PB1F2-NS2/NEP, NS2/NEP-PB1F2-K-HA, PB1F2-NS2/NEP-K-HA, HA-NS2/NEP-K-PB1F2, HA-PB1F2-K-NS2/NEP, NS2/NEP-K-PB1F2-HA, PB1F2-K-NS2/NEP-HA, HA-K-NS2/NEP-K-PB1F2, HA-K-PB1F2-K-NS2/NEP, NS2/NEP-K-PB1F2-K-HA, PB1F2-K-NS2/NEP-K-HA, HA-C-NS2/NEP-PB1F2, HA-C-PB1F2-NS2/NEP, NS2/NEP-PB1F2-C-HA, PB1F2-NS2/NEP-C-HA, HA-NS2/NEP-C-PB1F2, HA-PB1F2-C-NS2/NEP, NS2/NEP-C-PB1F2-HA, PB1F2-C-NS2/NEP-HA, HA-C-NS2/NEP-C-PB1F2, HA-C-PB1F2-C-NS2/NEP, NS2/NEP-C-PB1F2-C-HA, PB1F2-C-NS2/NEP-C-HA, HA-K-NS2/NEP-C-PB1F2, HA-K-PB1F2-C-NS2/NEP, NS2/NEP-C-PB1F2-K-HA, PB1F2-C-NS2/NEP-K-HA, HA-C-NS2/NEP-K-PB1F2, HA-C-PB1F2-K-NS2/NEP, NS2/NEP-K-PB1F2-C-HA, PB1F2-K-NS2/NEP-C-HA, HA-K-PA, PA-K-HA, HA-K-PA-PA, PA-PA-K-HA, HA-PA-K-PA, PA-K-PA-HA, HA-K-PA-K-PA, PA-K-PA-K-HA, HA-C-PA, PA-C-HA, HA-C-PA-PA, PA-PA-C-HA, HA-PA-C-PA, PA-C-PA-HA, HA-C-PA-C-PA, PA-C-PA-C-HA, HA-K-PA-C-PA, PA-C-PA-K-HA, HA-C-PA-K-PA, PA-K-PA-C-HA, HA-K-PA-PB1, HA-K-PB1-PA, PA-PB1-K-HA, PB1-PA-K-HA, HA-PA-K-PB1, HA-PB1-K-PA, PA-K-PB1-HA, PB1-K-PA-HA, HA-K-PA-K-PB1, HA-K-PB1-K-PA, PA-K-PB1-K-HA, PB1-K-PA-K-HA, HA-C-PA-PB1, HA-C-PB1-PA,

PA-PB1-C-HA, PB1-PA-C-HA, HA-PA-C-PB1, HA-PB1-C-PA, PA-C-PB1-HA, PB1-C-PA-HA, HA-C-PA-C-PB1, HA-C-PB1-C-PA, PA-C-PB1-C-HA, PB1-C-PA-C-HA, HA-K-PA-C-PB1, HA-K-PB1-C-PA, PA-C-PB1-K-HA, PB1-C-PA-K-HA, HA-C-PA-K-PB1, HA-C-PB1-K-PA, PA-K-PB1-C-HA, PB1-K-PA-C-HA, HA-K-PA-PB2, HA-K-PB2-PA, PA-PB2-K-HA, PB2-PA-K-HA, HA-PA-K-PB2, HA-PB2-K-PA, PA-K-PB2-HA, PB2-K-PA-HA, HA-K-PA-K-PB2, HA-K-PB2-K-PA, PA-K-PB2-K-HA, PB2-K-PA-K-HA, HA-C-PA-PB2, HA-C-PB2-PA, PA-PB2-C-HA, PB2-PA-C-HA, HA-PA-C-PB2, HA-PB2-C-PA, PA-C-PB2-HA, PB2-C-PA-HA, HA-C-PA-C-PB2, HA-C-PB2-C-PA, PA-C-PB2-C-HA, PB2-C-PA-C-HA, HA-K-PA-C-PB2, HA-K-PB2-C-PA, PA-C-PB2-K-HA, PB2-C-PA-K-HA, HA-C-PA-K-PB2, HA-C-PB2-K-PA, PA-K-PB2-C-HA, PB2-K-PA-C-HA, HA-K-PA-PB1F2, HA-K-PB1F2-PA, PA-PB1F2-K-HA, PB1F2-PA-K-HA, HA-PA-K-PB1F2, HA-PB1F2-K-PA, PA-K-PB1F2-HA, PB1F2-K-PA-HA, HA-K-PA-K-PB1F2, HA-K-PB1F2-K-PA, PA-K-PB1F2-K-HA, PB1F2-K-PA-K-HA, HA-C-PA-PB1F2, HA-C-PB1F2-PA, PA-PB1F2-C-HA, PB1F2-PA-C-HA, HA-PA-C-PB1F2, HA-PB1F2-C-PA, PA-C-PB1F2-HA, PB1F2-C-PA-HA, HA-C-PA-C-PB1F2, HA-C-PB1F2-C-PA, PA-C-PB1F2-C-HA, PB1F2-C-PA-C-HA, HA-K-PA-C-PB1F2, HA-K-PB1F2-C-PA, PA-C-PB1F2-K-HA, PB1F2-C-PA-K-HA, HA-C-PA-K-PB1F2, HA-C-PB1F2-K-PA, PA-K-PB1F2-C-HA, PB1F2-K-PA-C-HA, HA-K-PB1, PB1-K-HA, HA-K-PB1-PB1, PB1-PB1-K-HA, HA-PB1-K-PB1, PB1-K-PB1-HA, HA-K-PB1-K-PB1, PB1-K-PB1-K-HA, HA-C-PB1, PB1-C-HA, HA-C-PB1-PB1, PB1-PB1-C-HA, HA-PB1-C-PB1, PB1-C-PB1-HA, HA-C-PB1-C-PB1, PB1-C-PB1-C-HA, HA-K-PB1-C-PB1, PB1-C-PB1-K-HA, HA-C-PB1-K-PB1, PB1-K-PB1-C-HA, HA-K-PB1-PB2, HA-K-PB2-PB1, PB1-PB2-K-HA, PB2-PB1-K-HA, HA-PB1-K-PB2, HA-PB2-K-PB1, PB1-K-PB2-HA, PB2-K-PB1-HA, HA-K-PB1-K-PB2, HA-K-PB2-K-PB1, PB1-K-PB2-K-HA, PB2-K-PB1-K-HA, HA-C-PB1-PB2, HA-C-PB2-PB1, PB1-PB2-C-HA, PB2-PB1-C-HA, HA-PB1-C-PB2, HA-PB2-C-PB1, PB1-C-PB2-HA, PB2-C-PB1-HA, HA-C-PB1-C-PB2, HA-C-PB2-C-PB1, PB1-C-PB2-C-HA, PB2-C-PB1-C-HA, HA-K-PB1-C-PB2, HA-K-PB2-C-PB1, PB1-C-PB2-K-HA, PB2-C-PB1-K-HA, HA-C-PB1-K-PB2, HA-C-PB2-K-PB1, PB1-K-PB2-C-HA, PB2-K-PB1-C-HA, HA-K-PB1-PB1F2, HA-K-PB1F2-PB1, PB1-PB1F2-K-HA, PB1F2-PB1-K-HA, HA-PB1-K-PB1F2, HA-PB1F2-K-PB1, PB1-K-PB1F2-HA, PB1F2-K-PB1-HA, HA-K-PB1-K-PB1F2, HA-K-PB1F2-K-PB1, PB1-K-PB1F2-K-HA, PB1F2-K-PB1-K-HA, HA-C-PB1-PB1F2, HA-C-PB1F2-PB1, PB1-PB1F2-C-HA, PB1F2-PB1-C-HA, HA-PB1-C-PB1F2, HA-PB1F2-C-PB1, PB1-C-PB1F2-HA, PB1F2-C-PB1-HA, HA-C-PB1-C-PB1F2, HA-C-PB1F2-C-PB1, PB1-C-PB1F2-C-HA, PB1F2-C-PB1-C-HA, HA-K-PB1-C-PB1F2, HA-K-PB1F2-C-PB1, PB1-C-PB1F2-K-HA, PB1F2-C-PB1-K-HA, HA-C-PB1-K-PB1F2, HA-C-PB1F2-K-PB1, PB1-K-PB1F2-C-HA, PB1F2-K-PB1-C-HA, HA-K-PB2, PB2-K-HA, HA-K-PB2-PB2, PB2-PB2-K-HA, HA-PB2-K-PB2, PB2-K-PB2-HA, HA-K-PB2-K-PB2, PB2-K-PB2-

K-HA, HA-C-PB2, PB2-C-HA, HA-C-PB2-PB2, PB2-PB2-C-HA, HA-PB2-C-PB2, PB2-C-PB2-HA, HA-C-PB2-C-PB2, PB2-C-PB2-C-HA, HA-K-PB2-C-PB2, PB2-C-PB2-K-HA, HA-C-PB2-K-PB2, PB2-K-PB2-C-HA, HA-K-PB2-PB1F2, HA-K-PB1F2-PB2, PB2-PB1F2-K-HA, PB1F2-PB2-K-HA, HA-PB2-K-PB1F2, HA-PB1F2-K-PB2, PB2-K-PB1F2-HA, PB1F2-K-PB2-HA, HA-K-PB2-K-PB1F2, HA-K-PB1F2-K-PB2, PB2-K-PB1F2-K-HA, PB1F2-K-PB2-K-HA, HA-C-PB2-PB1F2, HA-C-PB1F2-PB2, PB2-PB1F2-C-HA, PB1F2-PB2-C-HA, HA-PB2-C-PB1F2, HA-PB1F2-C-PB2, PB2-C-PB1F2-HA, PB1F2-C-PB2-HA, HA-C-PB2-C-PB1F2, HA-C-PB1F2-C-PB2, PB2-C-PB1F2-C-HA, PB1F2-C-PB2-C-HA, HA-K-PB2-C-PB1F2, HA-K-PB1F2-C-PB2, PB2-C-PB1F2-K-HA, PB1F2-C-PB2-K-HA, HA-C-PB2-K-PB1F2, HA-C-PB1F2-K-PB2, PB2-K-PB1F2-C-HA, PB1F2-K-PB2-C-HA, HA-K-PB1F2, PB1F2-K-HA, HA-K-PB1F2-PB1F2, PB1F2-PB1F2-K-HA, HA-PB1F2-K-PB1F2, PB1F2-K-PB1F2-HA, HA-K-PB1F2-K-PB1F2, PB1F2-K-PB1F2-K-HA, HA-C-PB1F2, PB1F2-C-HA, HA-C-PB1F2-PB1F2, PB1F2-PB1F2-C-HA, HA-PB1F2-C-PB1F2, PB1F2-C-PB1F2-HA, HA-C-PB1F2-C-PB1F2, PB1F2-C-PB1F2-C-HA, HA-K-PB1F2-C-PB1F2, PB1F2-C-PB1F2-K-HA, HA-C-PB1F2-K-PB1F2, PB1F2-K-PB1F2-C-HA, NA-K-NP, NP-K-NA, NA-K-NP-NP, NP-NP-K-NA, NA-NP-K-NP, NP-K-NP-NA, NA-K-NP-K-NP, NP-K-NP-K-NA, NA-C-NP, NP-C-NA, NA-C-NP-NP, NP-NP-C-NA, NA-NP-C-NP, NP-C-NP-NA, NA-C-NP-C-NP, NP-C-NP-C-NA, NA-K-NP-C-NP, NP-C-NP-K-NA, NA-C-NP-K-NP, NP-K-NP-C-NA, NA-K-NP-M1, NA-K-M1-NP, NP-M1-K-NA, M1-NP-K-NA, NA-NP-K-M1, NA-M1-K-NP, NP-K-M1-NA, M1-K-NP-NA, NA-K-NP-K-M1, NA-K-M1-K-NP, NP-K-M1-K-NA, M1-K-NP-K-NA, NA-C-NP-M1, NA-C-M1-NP, NP-M1-C-NA, M1-NP-C-NA, NA-NP-C-M1, NA-M1-C-NP, NP-C-M1-NA, M1-C-NP-NA, NA-C-NP-C-M1, NA-C-M1-C-NP, NP-C-M1-C-NA, M1-C-NP-C-NA, NA-K-NP-C-M1, NA-K-M1-C-NP, NP-C-M1-K-NA, M1-C-NP-K-NA, NA-C-NP-K-M1, NA-C-M1-K-NP, NP-K-M1-C-NA, M1-K-NP-C-NA, NA-K-NP-M2, NA-K-M2-NP, NP-M2-K-NA, M2-NP-K-NA, NA-NP-K-M2, NA-M2-K-NP, NP-K-M2-NA, M2-K-NP-NA, NA-K-NP-K-M2, NA-K-M2-K-NP, NP-K-M2-K-NA, M2-K-NP-K-NA, NA-C-NP-M2, NA-C-M2-NP, NP-M2-C-NA, M2-NP-C-NA, NA-NP-C-M2, NA-M2-C-NP, NP-C-M2-NA, M2-C-NP-NA, NA-C-NP-C-M2, NA-C-M2-C-NP, NP-C-M2-C-NA, M2-C-NP-C-NA, NA-K-NP-C-M2, NA-K-M2-C-NP, NP-C-M2-K-NA, M2-C-NP-K-NA, NA-C-NP-K-M2, NA-C-M2-K-NP, NP-K-M2-C-NA, M2-K-NP-C-NA, NA-K-NP-NS1, NA-K-NS1-NP, NP-NS1-K-NA, NS1-NP-K-NA, NA-NP-K-NS1, NA-NS1-K-NP, NP-K-NS1-NA, NS1-K-NP-NA, NA-K-NP-K-NS1, NA-K-NS1-K-NP, NP-K-NS1-K-NA, NS1-K-NP-K-NA, NA-C-NP-NS1, NA-C-NS1-NP, NP-NS1-C-NA, NS1-NP-C-NA, NA-NP-C-NS1, NA-NS1-C-NP, NP-C-NS1-NA, NS1-C-NP-NA, NA-C-NP-C-NS1, NA-C-NS1-C-NP, NP-C-NS1-C-NA, NS1-C-NP-C-NA, NA-K-NP-C-NS1, NA-K-NS1-C-NP, NP-C-NS1-K-NA, NS1-C-NP-K-NA, NA-C-NP-K-NS1, NA-C-NS1-K-NP, NP-K-NS1-C-NA, NS1-

K-NP-C-NA, NA-K-NP-NS2/NEP, NA-K-NS2/NEP-NP, NP-NS2/NEP-K-NA, NS2/NEP-NP-K-NA, NA-NP-K-NS2/NEP, NA-NS2/NEP-K-NP, NP-K-NS2/NEP-NA, NS2/NEP-K-NP-NA, NA-K-NP-K-NS2/NEP, NA-K-NS2/NEP-K-NP, NP-K-NS2/NEP-K-NA, NS2/NEP-K-NP-K-NA, NA-C-NP-NS2/NEP, NA-C-NS2/NEP-NP, NP-NS2/NEP-C-NA, NS2/NEP-NP-C-NA, NA-NP-C-NS2/NEP, NA-NS2/NEP-C-NP, NP-C-NS2/NEP-NA, NS2/NEP-C-NP-NA, NA-C-NP-C-NS2/NEP, NA-C-NS2/NEP-C-NP, NP-C-NS2/NEP-C-NA, NS2/NEP-C-NP-C-NA, NA-K-NP-C-NS2/NEP, NA-K-NS2/NEP-C-NP, NP-C-NS2/NEP-K-NA, NS2/NEP-C-NP-K-NA, NA-C-NP-K-NS2/NEP, NA-C-NS2/NEP-K-NP, NP-K-NS2/NEP-C-NA, NS2/NEP-K-NP-C-NA, NA-K-NP-PA, NA-K-PA-NP, NP-PA-K-NA, PA-NP-K-NA, NA-NP-K-PA, NA-PA-K-NP, NP-K-PA-NA, PA-K-NP-NA, NA-K-NP-K-PA, NA-K-PA-K-NP, NP-K-PA-K-NA, PA-K-NP-K-NA, NA-C-NP-PA, NA-C-PA-NP, NP-PA-C-NA, PA-NP-C-NA, NA-NP-C-PA, NA-PA-C-NP, NP-C-PA-NA, PA-C-NP-NA, NA-C-NP-C-PA, NA-C-PA-C-NP, NP-C-PA-C-NA, PA-C-NP-C-NA, NA-K-NP-C-PA, NA-K-PA-C-NP, NP-C-PA-K-NA, PA-C-NP-K-NA, NA-C-NP-K-PA, NA-C-PA-K-NP, NP-K-PA-C-NA, PA-K-NP-C-NA, NA-K-NP-PB1, NA-K-PB1-NP, NP-PB1-K-NA, PB1-NP-K-NA, NA-NP-K-PB1, NA-PB1-K-NP, NP-K-PB1-NA, PB1-K-NP-NA, NA-K-NP-K-PB1, NA-K-PB1-K-NP, NP-K-PB1-K-NA, PB1-K-NP-K-NA, NA-C-NP-PB1, NA-C-PB1-NP, NP-PB1-C-NA, PB1-NP-C-NA, NA-NP-C-PB1, NA-PB1-C-NP, NP-C-PB1-NA, PB1-C-NP-NA, NA-C-NP-C-PB1, NA-C-PB1-C-NP, NP-C-PB1-C-NA, PB1-C-NP-C-NA, NA-K-NP-C-PB1, NA-K-PB1-C-NP, NP-C-PB1-K-NA, PB1-C-NP-K-NA, NA-C-NP-K-PB1, NA-C-PB1-K-NP, NP-K-PB1-C-NA, PB1-K-NP-C-NA, NA-K-NP-PB2, NA-K-PB2-NP, NP-PB2-K-NA, PB2-NP-K-NA, NA-NP-K-PB2, NA-PB2-K-NP, NP-K-PB2-NA, PB2-K-NP-NA, NA-K-NP-K-PB2, NA-K-PB2-K-NP, NP-K-PB2-K-NA, PB2-K-NP-K-NA, NA-C-NP-PB2, NA-C-PB2-NP, NP-PB2-C-NA, PB2-NP-C-NA, NA-NP-C-PB2, NA-PB2-C-NP, NP-C-PB2-NA, PB2-C-NP-NA, NA-C-NP-C-PB2, NA-C-PB2-C-NP, NP-C-PB2-C-NA, PB2-C-NP-C-NA, NA-K-NP-C-PB2, NA-K-PB2-C-NP, NP-C-PB2-K-NA, PB2-C-NP-K-NA, NA-C-NP-K-PB2, NA-C-PB2-K-NP, NP-K-PB2-C-NA, PB2-K-NP-C-NA, NA-K-NP-PB1F2, NA-K-PB1F2-NP, NP-PB1F2-K-NA, PB1F2-NP-K-NA, NA-NP-K-PB1F2, NA-PB1F2-K-NP, NP-K-PB1F2-NA, PB1F2-K-NP-NA, NA-K-NP-K-PB1F2, NA-K-PB1F2-K-NP, NP-K-PB1F2-K-NA, PB1F2-K-NP-K-NA, NA-C-NP-PB1F2, NA-C-PB1F2-NP, NP-PB1F2-C-NA, PB1F2-NP-C-NA, NA-NP-C-PB1F2, NA-PB1F2-C-NP, NP-C-PB1F2-NA, PB1F2-C-NP-NA, NA-C-NP-C-PB1F2, NA-C-PB1F2-C-NP, NP-C-PB1F2-C-NA, PB1F2-C-NP-C-NA, NA-K-NP-C-PB1F2, NA-K-PB1F2-C-NP, NP-C-PB1F2-K-NA, PB1F2-C-NP-K-NA, NA-C-NP-K-PB1F2, NA-C-PB1F2-K-NP, NP-K-PB1F2-C-NA, PB1F2-K-NP-C-NA, NA-K-M1, M1-K-NA, NA-K-M1-M1, M1-M1-K-NA, NA-M1-K-M1, M1-K-M1-NA, NA-K-M1-K-M1, M1-K-M1-K-NA, NA-C-M1, M1-C-NA, NA-C-M1-M1, M1-M1-C-NA, NA-M1-C-M1, M1-C-M1-NA, NA-C-M1-C-

M1, M1-C-M1-C-NA, NA-K-M1-C-M1, M1-C-M1-K-NA, NA-C-M1-K-M1, M1-K-M1-C-NA, NA-K-M1-M2, NA-K-M2-M1, M1-M2-K-NA, M2-M1-K-NA, NA-M1-K-M2, NA-M2-K-M1, M1-K-M2-NA, M2-K-M1-NA, NA-K-M1-K-M2, NA-K-M2-K-M1, M1-K-M2-K-NA, M2-K-M1-K-NA, NA-C-M1-M2, NA-C-M2-M1, M1-M2-C-NA, M2-M1-C-NA, NA-M1-C-M2, NA-M2-C-M1, M1-C-M2-NA, M2-C-M1-NA, NA-C-M1-C-M2, NA-C-M2-C-M1, M1-C-M2-C-NA, M2-C-M1-C-NA, NA-K-M1-C-M2, NA-K-M2-C-M1, M1-C-M2-K-NA, M2-C-M1-K-NA, NA-C-M1-K-M2, NA-C-M2-K-M1, M1-K-M2-C-NA, M2-K-M1-C-NA, NA-K-M1-NS1, NA-K-NS1-M1, M1-NS1-K-NA, NS1-M1-K-NA, NA-M1-K-NS1, NA-NS1-K-M1, M1-K-NS1-NA, NS1-K-M1-NA, NA-K-M1-K-NS1, NA-K-NS1-K-M1, M1-K-NS1-K-NA, NS1-K-M1-K-NA, NA-C-M1-NS1, NA-C-NS1-M1, M1-NS1-C-NA, NS1-M1-C-NA, NA-M1-C-NS1, NA-NS1-C-M1, M1-C-NS1-NA, NS1-C-M1-NA, NA-C-M1-C-NS1, NA-C-NS1-C-M1, M1-C-NS1-C-NA, NS1-C-M1-C-NA, NA-K-M1-C-NS1, NA-K-NS1-C-M1, M1-C-NS1-K-NA, NS1-C-M1-K-NA, NA-C-M1-K-NS1, NA-C-NS1-K-M1, M1-K-NS1-C-NA, NS1-K-M1-C-NA, NA-K-M1-NS2/NEP, NA-K-NS2/NEP-M1, M1-NS2/NEP-K-NA, NS2/NEP-M1-K-NA, NA-M1-K-NS2/NEP, NA-NS2/NEP-K-M1, M1-K-NS2/NEP-NA, NS2/NEP-K-M1-NA, NA-K-M1-K-NS2/NEP, NA-K-NS2/NEP-K-M1, M1-K-NS2/NEP-K-NA, NS2/NEP-K-M1-K-NA, NA-C-M1-NS2/NEP, NA-C-NS2/NEP-M1, M1-NS2/NEP-C-NA, NS2/NEP-M1-C-NA, NA-M1-C-NS2/NEP, NA-NS2/NEP-C-M1, M1-C-NS2/NEP-NA, NS2/NEP-C-M1-NA, NA-C-M1-C-NS2/NEP, NA-C-NS2/NEP-C-M1, M1-C-NS2/NEP-C-NA, NS2/NEP-C-M1-C-NA, NA-K-M1-C-NS2/NEP, NA-K-NS2/NEP-C-M1, M1-C-NS2/NEP-K-NA, NS2/NEP-C-M1-K-NA, NA-C-M1-K-NS2/NEP, NA-C-NS2/NEP-K-M1, M1-K-NS2/NEP-C-NA, NS2/NEP-K-M1-C-NA, NA-K-M1-PA, NA-K-PA-M1, M1-PA-K-NA, PA-M1-K-NA, NA-M1-K-PA, NA-PA-K-M1, M1-K-PA-NA, PA-K-M1-NA, NA-K-M1-K-PA, NA-K-PA-K-M1, M1-K-PA-K-NA, PA-K-M1-K-NA, NA-C-M1-PA, NA-C-PA-M1, M1-PA-C-NA, PA-M1-C-NA, NA-M1-C-PA, NA-PA-C-M1, M1-C-PA-NA, PA-C-M1-NA, NA-C-M1-C-PA, NA-C-PA-C-M1, M1-C-PA-C-NA, PA-C-M1-C-NA, NA-K-M1-C-PA, NA-K-PA-C-M1, M1-C-PA-K-NA, PA-C-M1-K-NA, NA-C-M1-K-PA, NA-C-PA-K-M1, M1-K-PA-C-NA, PA-K-M1-C-NA, NA-K-M1-PB1, NA-K-PB1-M1, M1-PB1-K-NA, PB1-M1-K-NA, NA-M1-K-PB1, NA-PB1-K-M1, M1-K-PB1-NA, PB1-K-M1-NA, NA-K-M1-K-PB1, NA-K-PB1-K-M1, M1-K-PB1-K-NA, PB1-K-M1-K-NA, NA-C-M1-PB1, NA-C-PB1-M1, M1-PB1-C-NA, PB1-M1-C-NA, NA-M1-C-PB1, NA-PB1-C-M1, M1-C-PB1-NA, PB1-C-M1-NA, NA-C-M1-C-PB1, NA-C-PB1-C-M1, M1-C-PB1-C-NA, PB1-C-M1-C-NA, NA-K-M1-C-PB1, NA-K-PB1-C-M1, M1-C-PB1-K-NA, PB1-C-M1-K-NA, NA-C-M1-K-PB1, NA-C-PB1-K-M1, M1-K-PB1-C-NA, PB1-K-M1-C-NA, NA-K-M1-PB2, NA-K-PB2-M1, M1-PB2-K-NA, PB2-M1-K-NA, NA-M1-K-PB2, NA-PB2-K-M1, M1-K-PB2-NA, PB2-K-M1-NA, NA-K-M1-K-PB2, NA-K-PB2-K-M1, M1-K-PB2-K-NA, PB2-K-M1-K-

NA, NA-C-M1-PB2, NA-C-PB2-M1, M1-PB2-C-NA, PB2-M1-C-NA, NA-M1-C-PB2, NA-PB2-C-M1, M1-C-PB2-NA, PB2-C-M1-NA, NA-C-M1-C-PB2, NA-C-PB2-C-M1, M1-C-PB2-C-NA, PB2-C-M1-C-NA, NA-K-M1-C-PB2, NA-K-PB2-C-M1, M1-C-PB2-K-NA, PB2-C-M1-K-NA, NA-C-M1-K-PB2, NA-C-PB2-K-M1, M1-K-PB2-C-NA, PB2-K-M1-C-NA, NA-K-M1-PB1F2, NA-K-PB1F2-M1, M1-PB1F2-K-NA, PB1F2-M1-K-NA, NA-M1-K-PB1F2, NA-PB1F2-K-M1, M1-K-PB1F2-NA, PB1F2-K-M1-NA, NA-K-M1-K-PB1F2, NA-K-PB1F2-K-M1, M1-K-PB1F2-K-NA, PB1F2-K-M1-K-NA, NA-C-M1-PB1F2, NA-C-PB1F2-M1, M1-PB1F2-C-NA, PB1F2-M1-C-NA, NA-M1-C-PB1F2, NA-PB1F2-C-M1, M1-C-PB1F2-NA, PB1F2-C-M1-NA, NA-C-M1-C-PB1F2, NA-C-PB1F2-C-M1, M1-C-PB1F2-C-NA, PB1F2-C-M1-C-NA, NA-K-M1-C-PB1F2, NA-K-PB1F2-C-M1, M1-C-PB1F2-K-NA, PB1F2-C-M1-K-NA, NA-C-M1-K-PB1F2, NA-C-PB1F2-K-M1, M1-K-PB1F2-C-NA, PB1F2-K-M1-C-NA, NA-K-M2, M2-K-NA, NA-K-M2-M2, M2-M2-K-NA, NA-M2-K-M2, M2-K-M2-NA, NA-K-M2-K-M2, M2-K-M2-K-NA, NA-C-M2, M2-C-NA, NA-C-M2-M2, M2-M2-C-NA, NA-M2-C-M2, M2-C-M2-NA, NA-C-M2-C-M2, M2-C-M2-C-NA, NA-K-M2-C-M2, M2-C-M2-K-NA, NA-C-M2-K-M2, M2-K-M2-C-NA, NA-K-M2-NS1, NA-K-NS1-M2, M2-NS1-K-NA, NS1-M2-K-NA, NA-M2-K-NS1, NA-NS1-K-M2, M2-K-NS1-NA, NS1-K-M2-NA, NA-K-M2-K-NS1, NA-K-NS1-K-M2, M2-K-NS1-K-NA, NS1-K-M2-K-NA, NA-C-M2-NS1, NA-C-NS1-M2, M2-NS1-C-NA, NS1-M2-C-NA, NA-M2-C-NS1, NA-NS1-C-M2, M2-C-NS1-NA, NS1-C-M2-NA, NA-C-M2-C-NS1, NA-C-NS1-C-M2, M2-C-NS1-C-NA, NS1-C-M2-C-NA, NA-K-M2-C-NS1, NA-K-NS1-C-M2, M2-C-NS1-K-NA, NS1-C-M2-K-NA, NA-C-M2-K-NS1, NA-C-NS1-K-M2, M2-K-NS1-C-NA, NS1-K-M2-C-NA, NA-K-M2-NS2/NEP, NA-K-NS2/NEP-M2, M2-NS2/NEP-K-NA, NS2/NEP-M2-K-NA, NA-M2-K-NS2/NEP, NA-NS2/NEP-K-M2, M2-K-NS2/NEP-NA, NS2/NEP-K-M2-NA, NA-K-M2-K-NS2/NEP, NA-K-NS2/NEP-K-M2, M2-K-NS2/NEP-K-NA, NS2/NEP-K-M2-K-NA, NA-C-M2-NS2/NEP, NA-C-NS2/NEP-M2, M2-NS2/NEP-C-NA, NS2/NEP-M2-C-NA, NA-M2-C-NS2/NEP, NA-NS2/NEP-C-M2, M2-C-NS2/NEP-NA, NS2/NEP-C-M2-NA, NA-C-M2-C-NS2/NEP, NA-C-NS2/NEP-C-M2, M2-C-NS2/NEP-C-NA, NS2/NEP-C-M2-C-NA, NA-K-M2-C-NS2/NEP, NA-K-NS2/NEP-C-M2, M2-C-NS2/NEP-K-NA, NS2/NEP-C-M2-K-NA, NA-C-M2-K-NS2/NEP, NA-C-NS2/NEP-K-M2, M2-K-NS2/NEP-C-NA, NS2/NEP-K-M2-C-NA, NA-K-M2-PA, NA-K-PA-M2, M2-PA-K-NA, PA-M2-K-NA, NA-M2-K-PA, NA-PA-K-M2, M2-K-PA-NA, PA-K-M2-NA, NA-K-M2-K-PA, NA-K-PA-K-M2, M2-K-PA-K-NA, PA-K-M2-K-NA, NA-C-M2-PA, NA-C-PA-M2, M2-PA-C-NA, PA-M2-C-NA, NA-M2-C-PA, NA-PA-C-M2, M2-C-PA-NA, PA-C-M2-NA, NA-C-M2-C-PA, NA-C-PA-C-M2, M2-C-PA-C-NA, PA-C-M2-C-NA, NA-K-M2-C-PA, NA-K-PA-C-M2, M2-C-PA-K-NA, PA-C-M2-K-NA, NA-C-M2-K-PA, NA-C-PA-K-M2, M2-K-PA-C-NA, PA-K-M2-C-NA, NA-K-M2-PB1, NA-K-PB1-M2, M2-PB1-K-NA, PB1-M2-K-NA, NA-M2-

K-PB1, NA-PB1-K-M2, M2-K-PB1-NA, PB1-K-M2-NA, NA-K-M2-K-PB1, NA-K-PB1-K-M2, M2-K-PB1-K-NA, PB1-K-M2-K-NA, NA-C-M2-PB1, NA-C-PB1-M2, M2-PB1-C-NA, PB1-M2-C-NA, NA-M2-C-PB1, NA-PB1-C-M2, M2-C-PB1-NA, PB1-C-M2-NA, NA-C-M2-C-PB1, NA-C-PB1-C-M2, M2-C-PB1-C-NA, PB1-C-M2-C-NA, NA-K-M2-C-PB1, NA-K-PB1-C-M2, M2-C-PB1-K-NA, PB1-C-M2-K-NA, NA-C-M2-K-PB1, NA-C-PB1-K-M2, M2-K-PB1-C-NA, PB1-K-M2-C-NA, NA-K-M2-PB2, NA-K-PB2-M2, M2-PB2-K-NA, PB2-M2-K-NA, NA-M2-K-PB2, NA-PB2-K-M2, M2-K-PB2-NA, PB2-K-M2-NA, NA-K-M2-K-PB2, NA-K-PB2-K-M2, M2-K-PB2-K-NA, PB2-K-M2-K-NA, NA-C-M2-PB2, NA-C-PB2-M2, M2-PB2-C-NA, PB2-M2-C-NA, NA-M2-C-PB2, NA-PB2-C-M2, M2-C-PB2-NA, PB2-C-M2-NA, NA-C-M2-C-PB2, NA-C-PB2-C-M2, M2-C-PB2-C-NA, PB2-C-M2-C-NA, NA-K-M2-C-PB2, NA-K-PB2-C-M2, M2-C-PB2-K-NA, PB2-C-M2-K-NA, NA-C-M2-K-PB2, NA-C-PB2-K-M2, M2-K-PB2-C-NA, PB2-K-M2-C-NA, NA-K-M2-PB1F2, NA-K-PB1F2-M2, M2-PB1F2-K-NA, PB1F2-M2-K-NA, NA-M2-K-PB1F2, NA-PB1F2-K-M2, M2-K-PB1F2-NA, PB1F2-K-M2-NA, NA-K-M2-K-PB1F2, NA-K-PB1F2-K-M2, M2-K-PB1F2-K-NA, PB1F2-K-M2-K-NA, NA-C-M2-PB1F2, NA-C-PB1F2-M2, M2-PB1F2-C-NA, PB1F2-M2-C-NA, NA-M2-C-PB1F2, NA-PB1F2-C-M2, M2-C-PB1F2-NA, PB1F2-C-M2-NA, NA-C-M2-C-PB1F2, NA-C-PB1F2-C-M2, M2-C-PB1F2-C-NA, PB1F2-C-M2-C-NA, NA-K-M2-C-PB1F2, NA-K-PB1F2-C-M2, M2-C-PB1F2-K-NA, PB1F2-C-M2-K-NA, NA-C-M2-K-PB1F2, NA-C-PB1F2-K-M2, M2-K-PB1F2-C-NA, PB1F2-K-M2-C-NA, NA-K-NS1, NS1-K-NA, NA-K-NS1-NS1, NS1-NS1-K-NA, NA-NS1-K-NS1, NS1-K-NS1-NA, NA-K-NS1-K-NS1, NS1-K-NS1-K-NA, NA-C-NS1, NS1-C-NA, NA-C-NS1-NS1, NS1-NS1-C-NA, NA-NS1-C-NS1, NS1-C-NS1-NA, NA-C-NS1-C-NS1, NS1-C-NS1-C-NA, NA-K-NS1-C-NS1, NS1-C-NS1-K-NA, NA-C-NS1-K-NS1, NS1-K-NS1-C-NA, NA-K-NS1-NS2/NEP, NA-K-NS2/NEP-NS1, NS1-NS2/NEP-K-NA, NS2/NEP-NS1-K-NA, NA-NS1-K-NS2/NEP, NA-NS2/NEP-K-NS1, NS1-K-NS2/NEP-NA, NS2/NEP-K-NS1-NA, NA-K-NS1-K-NS2/NEP, NA-K-NS2/NEP-K-NS1, NS1-K-NS2/NEP-K-NA, NS2/NEP-K-NS1-K-NA, NA-C-NS1-NS2/NEP, NA-C-NS2/NEP-NS1, NS1-NS2/NEP-C-NA, NS2/NEP-NS1-C-NA, NA-NS1-C-NS2/NEP, NA-NS2/NEP-C-NS1, NS1-C-NS2/NEP-NA, NS2/NEP-C-NS1-NA, NA-C-NS1-C-NS2/NEP, NA-C-NS2/NEP-C-NS1, NS1-C-NS2/NEP-C-NA, NS2/NEP-C-NS1-C-NA, NA-K-NS1-C-NS2/NEP, NA-K-NS2/NEP-C-NS1, NS1-C-NS2/NEP-K-NA, NS2/NEP-C-NS1-K-NA, NA-C-NS1-K-NS2/NEP, NA-C-NS2/NEP-K-NS1, NS1-K-NS2/NEP-C-NA, NS2/NEP-K-NS1-C-NA, NA-K-NS1-PA, NA-K-PA-NS1, NS1-PA-K-NA, PA-NS1-K-NA, NA-NS1-K-PA, NA-PA-K-NS1, NS1-K-PA-NA, PA-K-NS1-NA, NA-K-NS1-K-PA, NA-K-PA-K-NS1, NS1-K-PA-K-NA, PA-K-NS1-K-NA, NA-C-NS1-PA, NA-C-PA-NS1, NS1-PA-C-NA, PA-NS1-C-NA, NA-NS1-C-PA, NA-PA-C-NS1, NS1-C-PA-NA, PA-C-NS1-NA, NA-C-NS1-C-PA, NA-C-PA-C-NS1, NS1-C-PA-C-NA, PA-C-NS1-C-NA, NA-K-

NS1-C-PA, NA-K-PA-C-NS1, NS1-C-PA-K-NA, PA-C-NS1-K-NA, NA-C-NS1-K-PA, NA-C-PA-K-NS1, NS1-K-PA-C-NA, PA-K-NS1-C-NA, NA-K-NS1-PB1, NA-K-PB1-NS1, NS1-PB1-K-NA, PB1-NS1-K-NA, NA-NS1-K-PB1, NA-PB1-K-NS1, NS1-K-PB1-NA, PB1-K-NS1-NA, NA-K-NS1-K-PB1, NA-K-PB1-K-NS1, NS1-K-PB1-K-NA, PB1-K-NS1-K-NA, NA-C-NS1-PB1, NA-C-PB1-NS1, NS1-PB1-C-NA, PB1-NS1-C-NA, NA-NS1-C-PB1, NA-PB1-C-NS1, NS1-C-PB1-NA, PB1-C-NS1-NA, NA-C-NS1-C-PB1, NA-C-PB1-C-NS1, NS1-C-PB1-C-NA, PB1-C-NS1-C-NA, NA-K-NS1-C-PB1, NA-K-PB1-C-NS1, NS1-C-PB1-K-NA, PB1-C-NS1-K-NA, NA-C-NS1-K-PB1, NA-C-PB1-K-NS1, NS1-K-PB1-C-NA, PB1-K-NS1-C-NA, NA-K-NS1-PB2, NA-K-PB2-NS1, NS1-PB2-K-NA, PB2-NS1-K-NA, NA-NS1-K-PB2, NA-PB2-K-NS1, NS1-K-PB2-NA, PB2-K-NS1-NA, NA-K-NS1-K-PB2, NA-K-PB2-K-NS1, NS1-K-PB2-K-NA, PB2-K-NS1-K-NA, NA-C-NS1-PB2, NA-C-PB2-NS1, NS1-PB2-C-NA, PB2-NS1-C-NA, NA-NS1-C-PB2, NA-PB2-C-NS1, NS1-C-PB2-NA, PB2-C-NS1-NA, NA-C-NS1-C-PB2, NA-C-PB2-C-NS1, NS1-C-PB2-C-NA, PB2-C-NS1-C-NA, NA-K-NS1-C-PB2, NA-K-PB2-C-NS1, NS1-C-PB2-K-NA, PB2-C-NS1-K-NA, NA-C-NS1-K-PB2, NA-C-PB2-K-NS1, NS1-K-PB2-C-NA, PB2-K-NS1-C-NA, NA-K-NS1-PB1F2, NA-K-PB1F2-NS1, NS1-PB1F2-K-NA, PB1F2-NS1-K-NA, NA-NS1-K-PB1F2, NA-PB1F2-K-NS1, NS1-K-PB1F2-NA, PB1F2-K-NS1-NA, NA-K-NS1-K-PB1F2, NA-K-PB1F2-K-NS1, NS1-K-PB1F2-K-NA, PB1F2-K-NS1-K-NA, NA-C-NS1-PB1F2, NA-C-PB1F2-NS1, NS1-PB1F2-C-NA, PB1F2-NS1-C-NA, NA-NS1-C-PB1F2, NA-PB1F2-C-NS1, NS1-C-PB1F2-NA, PB1F2-C-NS1-NA, NA-C-NS1-C-PB1F2, NA-C-PB1F2-C-NS1, NS1-C-PB1F2-C-NA, PB1F2-C-NS1-C-NA, NA-K-NS1-C-PB1F2, NA-K-PB1F2-C-NS1, NS1-C-PB1F2-K-NA, PB1F2-C-NS1-K-NA, NA-C-NS1-K-PB1F2, NA-C-PB1F2-K-NS1, NS1-K-PB1F2-C-NA, PB1F2-K-NS1-C-NA, NA-K-NS2/NEP, NS2/NEP-K-NA, NA-K-NS2/NEP-NS2/NEP, NS2/NEP-NS2/NEP-K-NA, NA-NS2/NEP-K-NS2/NEP, NS2/NEP-K-NS2/NEP-NA, NA-K-NS2/NEP-K-NS2/NEP, NS2/NEP-K-NS2/NEP-K-NA, NA-C-NS2/NEP, NS2/NEP-C-NA, NA-C-NS2/NEP-NS2/NEP, NS2/NEP-NS2/NEP-C-NA, NA-NS2/NEP-C-NS2/NEP, NS2/NEP-C-NS2/NEP-NA, NA-C-NS2/NEP-C-NS2/NEP, NS2/NEP-C-NS2/NEP-C-NA, NA-K-NS2/NEP-C-NS2/NEP, NS2/NEP-C-NS2/NEP-K-NA, NA-C-NS2/NEP-K-NS2/NEP, NS2/NEP-K-NS2/NEP-C-NA, NA-K-NS2/NEP-PA, NA-K-PA-NS2/NEP, NS2/NEP-PA-K-NA, PA-NS2/NEP-K-NA, NA-NS2/NEP-K-PA, NA-PA-K-NS2/NEP, NS2/NEP-K-PA-NA, PA-K-NS2/NEP-NA, NA-K-NS2/NEP-K-PA, NA-K-PA-K-NS2/NEP, NS2/NEP-K-PA-K-NA, PA-K-NS2/NEP-K-NA, NA-C-NS2/NEP-PA, NA-C-PA-NS2/NEP, NS2/NEP-PA-C-NA, PA-NS2/NEP-C-NA, NA-NS2/NEP-C-PA, NA-PA-C-NS2/NEP, NS2/NEP-C-PA-NA, PA-C-NS2/NEP-NA, NA-C-NS2/NEP-C-PA, NA-C-PA-C-NS2/NEP, NS2/NEP-C-PA-C-NA, PA-C-NS2/NEP-C-NA, NA-K-NS2/NEP-C-PA, NA-K-PA-C-NS2/NEP, NS2/NEP-C-PA-K-NA, PA-C-NS2/NEP-K-NA, NA-C-NS2/NEP-K-PA, NA-C-

PA-K-NS2/NEP, NS2/NEP-K-PA-C-NA, PA-K-NS2/NEP-C-NA, NA-K-NS2/NEP-PB1, NA-K-PB1-NS2/NEP, NS2/NEP-PB1-K-NA, PB1-NS2/NEP-K-NA, NA-NS2/NEP-K-PB1, NA-PB1-K-NS2/NEP, NS2/NEP-K-PB1-NA, PB1-K-NS2/NEP-NA, NA-K-NS2/NEP-K-PB1, NA-K-PB1-K-NS2/NEP, NS2/NEP-K-PB1-K-NA, PB1-K-NS2/NEP-K-NA, NA-C-NS2/NEP-PB1, NA-C-PB1-NS2/NEP, NS2/NEP-PB1-C-NA, PB1-NS2/NEP-C-NA, NA-NS2/NEP-C-PB1, NA-PB1-C-NS2/NEP, NS2/NEP-C-PB1-NA, PB1-C-NS2/NEP-NA, NA-C-NS2/NEP-C-PB1, NA-C-PB1-C-NS2/NEP, NS2/NEP-C-PB1-C-NA, PB1-C-NS2/NEP-C-NA, NA-K-NS2/NEP-C-PB1, NA-K-PB1-C-NS2/NEP, NS2/NEP-C-PB1-K-NA, PB1-C-NS2/NEP-K-NA, NA-C-NS2/NEP-K-PB1, NA-C-PB1-K-NS2/NEP, NS2/NEP-K-PB1-C-NA, PB1-K-NS2/NEP-C-NA, NA-K-NS2/NEP-PB2, NA-K-PB2-NS2/NEP, NS2/NEP-PB2-K-NA, PB2-NS2/NEP-K-NA, NA-NS2/NEP-K-PB2, NA-PB2-K-NS2/NEP, NS2/NEP-K-PB2-NA, PB2-K-NS2/NEP-NA, NA-K-NS2/NEP-K-PB2, NA-K-PB2-K-NS2/NEP, NS2/NEP-K-PB2-K-NA, PB2-K-NS2/NEP-K-NA, NA-C-NS2/NEP-PB2, NA-C-PB2-NS2/NEP, NS2/NEP-PB2-C-NA, PB2-NS2/NEP-C-NA, NA-NS2/NEP-C-PB2, NA-PB2-C-NS2/NEP, NS2/NEP-C-PB2-NA, PB2-C-NS2/NEP-NA, NA-C-NS2/NEP-C-PB2, NA-C-PB2-C-NS2/NEP, NS2/NEP-C-PB2-C-NA, PB2-C-NS2/NEP-C-NA, NA-K-NS2/NEP-C-PB2, NA-K-PB2-C-NS2/NEP, NS2/NEP-C-PB2-K-NA, PB2-C-NS2/NEP-K-NA, NA-C-NS2/NEP-K-PB2, NA-C-PB2-K-NS2/NEP, NS2/NEP-K-PB2-C-NA, PB2-K-NS2/NEP-C-NA, NA-K-NS2/NEP-PB1F2, NA-K-PB1F2-NS2/NEP, NS2/NEP-PB1F2-K-NA, PB1F2-NS2/NEP-K-NA, NA-NS2/NEP-K-PB1F2, NA-PB1F2-K-NS2/NEP, NS2/NEP-K-PB1F2-NA, PB1F2-K-NS2/NEP-NA, NA-K-NS2/NEP-K-PB1F2, NA-K-PB1F2-K-NS2/NEP, NS2/NEP-K-PB1F2-K-NA, PB1F2-K-NS2/NEP-K-NA, NA-C-NS2/NEP-PB1F2, NA-C-PB1F2-NS2/NEP, NS2/NEP-PB1F2-C-NA, PB1F2-NS2/NEP-C-NA, NA-NS2/NEP-C-PB1F2, NA-PB1F2-C-NS2/NEP, NS2/NEP-C-PB1F2-NA, PB1F2-C-NS2/NEP-NA, NA-C-NS2/NEP-C-PB1F2, NA-C-PB1F2-C-NS2/NEP, NS2/NEP-C-PB1F2-C-NA, PB1F2-C-NS2/NEP-C-NA, NA-K-NS2/NEP-C-PB1F2, NA-K-PB1F2-C-NS2/NEP, NS2/NEP-C-PB1F2-K-NA, PB1F2-C-NS2/NEP-K-NA, NA-C-NS2/NEP-K-PB1F2, NA-C-PB1F2-K-NS2/NEP, NS2/NEP-K-PB1F2-C-NA, PB1F2-K-NS2/NEP-C-NA, NA-K-PA, PA-K-NA, NA-K-PA-PA, PA-PA-K-NA, NA-PA-K-PA, PA-K-PA-NA, NA-K-PA-K-PA, PA-K-PA-K-NA, NA-C-PA, PA-C-NA, NA-C-PA-PA, PA-PA-C-NA, NA-PA-C-PA, PA-C-PA-NA, NA-C-PA-C-PA, PA-C-PA-C-NA, NA-K-PA-C-PA, PA-C-PA-K-NA, NA-C-PA-K-PA, PA-K-PA-C-NA, NA-K-PA-PB1, NA-K-PB1-PA, PA-PB1-K-NA, PB1-PA-K-NA, NA-PA-K-PB1, NA-PB1-K-PA, PA-K-PB1-NA, PB1-K-PA-NA, NA-K-PA-K-PB1, NA-K-PB1-K-PA, PA-K-PB1-K-NA, PB1-K-PA-K-NA, NA-C-PA-PB1, NA-C-PB1-PA, PA-PB1-C-NA, PB1-PA-C-NA, NA-PA-C-PB1, NA-PB1-C-PA, PA-C-PB1-NA, PB1-C-PA-NA, NA-C-PA-C-PB1, NA-C-PB1-C-PA, PA-C-PB1-C-NA, PB1-C-PA-C-NA, NA-K-PA-C-PB1, NA-K-PB1-C-PA, PA-C-PB1-K-NA, PB1-C-PA-K-

NA, NA-C-PA-K-PB1, NA-C-PB1-K-PA, PA-K-PB1-C-NA, PB1-K-PA-C-NA, NA-K-PA-PB2, NA-K-PB2-PA, PA-PB2-K-NA, PB2-PA-K-NA, NA-PA-K-PB2, NA-PB2-K-PA, PA-K-PB2-NA, PB2-K-PA-NA, NA-K-PA-K-PB2, NA-K-PB2-K-PA, PA-K-PB2-K-NA, PB2-K-PA-K-NA, NA-C-PA-PB2, NA-C-PB2-PA, PA-PB2-C-NA, PB2-PA-C-NA, NA-PA-C-PB2, NA-PB2-C-PA, PA-C-PB2-NA, PB2-C-PA-NA, NA-C-PA-C-PB2, NA-C-PB2-C-PA, PA-C-PB2-C-NA, PB2-C-PA-C-NA, NA-K-PA-C-PB2, NA-K-PB2-C-PA, PA-C-PB2-K-NA, PB2-C-PA-K-NA, NA-C-PA-K-PB2, NA-C-PB2-K-PA, PA-K-PB2-C-NA, PB2-K-PA-C-NA, NA-K-PA-PB1F2, NA-K-PB1F2-PA, PA-PB1F2-K-NA, PB1F2-PA-K-NA, NA-PA-K-PB1F2, NA-PB1F2-K-PA, PA-K-PB1F2-NA, PB1F2-K-PA-NA, NA-K-PA-K-PB1F2, NA-K-PB1F2-K-PA, PA-K-PB1F2-K-NA, PB1F2-K-PA-K-NA, NA-C-PA-PB1F2, NA-C-PB1F2-PA, PA-PB1F2-C-NA, PB1F2-PA-C-NA, NA-PA-C-PB1F2, NA-PB1F2-C-PA, PA-C-PB1F2-NA, PB1F2-C-PA-NA, NA-C-PA-C-PB1F2, NA-C-PB1F2-C-PA, PA-C-PB1F2-C-NA, PB1F2-C-PA-C-NA, NA-K-PA-C-PB1F2, NA-K-PB1F2-C-PA, PA-C-PB1F2-K-NA, PB1F2-C-PA-K-NA, NA-C-PA-K-PB1F2, NA-C-PB1F2-K-PA, PA-K-PB1F2-C-NA, PB1F2-K-PA-C-NA, NA-K-PB1, PB1-K-NA, NA-K-PB1-PB1, PB1-PB1-K-NA, NA-PB1-K-PB1, PB1-K-PB1-NA, NA-K-PB1-K-PB1, PB1-K-PB1-K-NA, NA-C-PB1, PB1-C-NA, NA-C-PB1-PB1, PB1-PB1-C-NA, NA-PB1-C-PB1, PB1-C-PB1-NA, NA-C-PB1-C-PB1, PB1-C-PB1-C-NA, NA-K-PB1-C-PB1, PB1-C-PB1-K-NA, NA-C-PB1-K-PB1, PB1-K-PB1-C-NA, NA-K-PB1-PB2, NA-K-PB2-PB1, PB1-PB2-K-NA, PB2-PB1-K-NA, NA-PB1-K-PB2, NA-PB2-K-PB1, PB1-K-PB2-NA, PB2-K-PB1-NA, NA-K-PB1-K-PB2, NA-K-PB2-K-PB1, PB1-K-PB2-K-NA, PB2-K-PB1-K-NA, NA-C-PB1-PB2, NA-C-PB2-PB1, PB1-PB2-C-NA, PB2-PB1-C-NA, NA-PB1-C-PB2, NA-PB2-C-PB1, PB1-C-PB2-NA, PB2-C-PB1-NA, NA-C-PB1-C-PB2, NA-C-PB2-C-PB1, PB1-C-PB2-C-NA, PB2-C-PB1-C-NA, NA-K-PB1-C-PB2, NA-K-PB2-C-PB1, PB1-C-PB2-K-NA, PB2-C-PB1-K-NA, NA-C-PB1-K-PB2, NA-C-PB2-K-PB1, PB1-K-PB2-C-NA, PB2-K-PB1-C-NA, NA-K-PB1-PB1F2, NA-K-PB1F2-PB1, PB1-PB1F2-K-NA, PB1F2-PB1-K-NA, NA-PB1-K-PB1F2, NA-PB1F2-K-PB1, PB1-K-PB1F2-NA, PB1F2-K-PB1-NA, NA-K-PB1-K-PB1F2, NA-K-PB1F2-K-PB1, PB1-K-PB1F2-K-NA, PB1F2-K-PB1-K-NA, NA-C-PB1-PB1F2, NA-C-PB1F2-PB1, PB1-PB1F2-C-NA, PB1F2-PB1-C-NA, NA-PB1-C-PB1F2, NA-PB1F2-C-PB1, PB1-C-PB1F2-NA, PB1F2-C-PB1-NA, NA-C-PB1-C-PB1F2, NA-C-PB1F2-C-PB1, PB1-C-PB1F2-C-NA, PB1F2-C-PB1-C-NA, NA-K-PB1-C-PB1F2, NA-K-PB1F2-C-PB1, PB1-C-PB1F2-K-NA, PB1F2-C-PB1-K-NA, NA-C-PB1-K-PB1F2, NA-C-PB1F2-K-PB1, PB1-K-PB1F2-C-NA, PB1F2-K-PB1-C-NA, NA-K-PB2, PB2-K-NA, NA-K-PB2-PB2, PB2-PB2-K-NA, NA-PB2-K-PB2, PB2-K-PB2-NA, NA-K-PB2-K-PB2, PB2-K-PB2-K-NA, NA-C-PB2, PB2-C-NA, NA-C-PB2-PB2, PB2-PB2-C-NA, NA-PB2-C-PB2, PB2-C-PB2-NA, NA-C-PB2-C-PB2, PB2-C-PB2-C-NA, NA-K-PB2-C-PB2, PB2-C-PB2-K-NA, NA-C-PB2-K-PB2, PB2-K-PB2-C-NA, NA-K-

PB2-PB1F2, NA-K-PB1F2-PB2, PB2-PB1F2-K-NA, PB1F2-PB2-K-NA, NA-PB2-K-PB1F2, NA-PB1F2-K-PB2, PB2-K-PB1F2-NA, PB1F2-K-PB2-NA, NA-K-PB2-K-PB1F2, NA-K-PB1F2-K-PB2, PB2-K-PB1F2-K-NA, PB1F2-K-PB2-K-NA, NA-C-PB2-PB1F2, NA-C-PB1F2-PB2, PB2-PB1F2-C-NA, PB1F2-PB2-C-NA, NA-PB2-C-PB1F2, NA-PB1F2-C-PB2, PB2-C-PB1F2-NA, PB1F2-C-PB2-NA, NA-C-PB2-C-PB1F2, NA-C-PB1F2-C-PB2, PB2-C-PB1F2-C-NA, PB1F2-C-PB2-C-NA, NA-K-PB2-C-PB1F2, NA-K-PB1F2-C-PB2, PB2-C-PB1F2-K-NA, PB1F2-C-PB2-K-NA, NA-C-PB2-K-PB1F2, NA-C-PB1F2-K-PB2, PB2-K-PB1F2-C-NA, PB1F2-K-PB2-C-NA, NA-K-PB1F2, PB1F2-K-NA, NA-K-PB1F2-PB1F2, PB1F2-PB1F2-K-NA, NA-PB1F2-K-PB1F2, PB1F2-K-PB1F2-NA, NA-K-PB1F2-K-PB1F2, PB1F2-K-PB1F2-K-NA, NA-C-PB1F2, PB1F2-C-NA, NA-C-PB1F2-PB1F2, PB1F2-PB1F2-C-NA, NA-PB1F2-C-PB1F2, PB1F2-C-PB1F2-NA, NA-C-PB1F2-C-PB1F2, PB1F2-C-PB1F2-C-NA, NA-K-PB1F2-C-PB1F2, PB1F2-C-PB1F2-K-NA, NA-C-PB1F2-K-PB1F2 or PB1F2-K-PB1F2-C-NA. Most preferably, the arrangement is NP-K-M1-C-HA.

It is within the scope of the present invention that every protein can be combined with any other protein and that any two proteins can or cannot be connected or linked by either a cleavage site or a linker peptide.

In preferred embodiments, the expression system is for use in the prophylaxis or treatment of viral infection, particularly preferably for use in the prophylaxis or treatment of an orthomyxovirus infection, preferably an influenza virus infection, more preferably an influenza A virus infection, and/or in the manufacturing of medicament for use in the prophylaxis or treatment of an orthomyxovirus infection, preferably an influenza virus infection, more preferably an influenza A virus infection, and/or for use in methods of prophylaxis or treatment of an orthomyxovirus infection, preferably an influenza virus infection, more preferably an influenza A virus infection, preferably an influenza virus infection, more preferably an influenza A virus infection.

In preferred embodiments, the expression system is for use in enhancing an immune response, preferably a B cell immune response an orthomyxovirus infection, preferably an influenza virus infection, more preferably an influenza A virus infection.

In a preferred embodiment of this aspect, HA is defined according to the eighth aspect.

It is particularly preferred that the viral polyprotein encoded by the first, the second and the third polynucleotide has an amino acid according to SEQ ID NO: 13 or a variant thereof and/or is encoded by a polynucleotide having the nucleic acid sequence of SEQ ID NO: 14 or a variant thereof. Preferably, the encoded triple antigen protein NP-M1-H1p is processed into a cytoplasmic NP-M1 fusion protein and a membrane spanning H1p protein by the 2A sequence.

In preferred embodiments, the expression system is for use in the prophylaxis or treatment of an orthomyxovirus infection, preferably an influenza A virus infection.

In more preferred embodiments, the expression system is for use in enhancing an immune response, preferably a B cell immune response against a an orthomyxovirus protein, preferably an influenza A virus protein.

In preferred embodiments, the vector or vectors comprising the first, and the second and/or the third polynucleotide is/are selected from the group consisting of plasmid, cosmid, phage, virus, and artificial chromosome. More preferably, a vector suitable for practicing the present invention is selected from the group consisting of plasmid vectors, cosmid vectors, phage vectors, preferably lambda phage and filamentous phage vectors, viral vectors, adenovirus vectors (e.g., non-replicating Ad5, Ad11, Ad26, Ad35, Ad49, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd 73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, and PanAd3 vectors or replication-competent Ad4 and Ad7 vectors), adeno-associated virus (AAV) vectors (e.g., AAV type 5 and type 2), alphavirus vectors (e.g., Venezuelan equine encephalitis virus (VEE), sindbis virus (SIN), semliki forest virus (SFV), and VEE-SIN chimeras), herpes virus vectors (e.g. vectors derived from cytomegaloviruses, like rhesus cytomegalovirus (RhCMV) (14)), arena virus vectors (e.g. lymphocytic choriomeningitis virus (LCMV) vectors (15)), measles virus vectors, pox virus vectors (e.g., vaccinia virus, modified vaccinia virus Ankara (MVA), NYVAC (derived from the Copenhagen strain of vaccinia), and avipox vectors: canarypox (ALVAC) and fowlpox (FPV) vectors), vesicular stomatitis virus vectors, retrovirus, lentivirus, viral like particles, and bacterial spores. The vectors ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63 and ChAd82 are described in detail in WO 2005/071093. The vectors PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146, and ChAd147 are described in detail in WO 2010/086189. It is particularly preferred that the vector is selected from the group consisting of MVA, ChAd63 and PanAd3.

In preferred embodiments, the expression system is for use in medicine. In more preferred embodiments, the expression system is for use in the prophylaxis or treatment of viral infection, particularly preferably for use in the prophylaxis or treatment of RSV infection.

In a second aspect, the present invention provides an isolated protein mixture encoded by the expression system of the first aspect. Preferably, the isolated protein mixture contains, essentially contains or comprises one or more of the viral proteins encoded by the expression system of the first aspect.

In preferred embodiments, the isolated protein mixture is for use in medicine. In particularly preferred embodiments, the isolated protein mixture is for use in the prophylaxis or treatment of viral infection, particularly preferably for use in the prophylaxis or treatment of RSV infection or in the prophylaxis or treatment of influenza A infection.

In a third aspect, the present invention provides an isolated host cell containing the expression system of the first aspect and/or the protein mixture of the second aspect. It is understood that such host cell includes but is not limited to prokaryotic (e.g. a bacterial cell) or eukaryotic cells (e.g. a fungal, plant or animal cell).

In preferred embodiments, the host cell is for use in medicine. In particularly preferred embodiments, the host cell is for use in the prophylaxis or treatment of viral infection, particularly preferably for use in the prophylaxis or treatment of RSV infection or in the prophylaxis or treatment of influenza A infection.

In a fourth aspect, the present invention provides a composition comprising the expression system of the first aspect or the protein mixture of the second aspect and a pharmaceutical acceptable carrier and/or excipient. Preferably, such composition is a pharmaceutical composition.

The composition of the fourth aspect contains a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

The compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

For preparing pharmaceutical compositions of the present invention, pharmaceutically acceptable carriers can be either solid or liquid.

Solid form compositions include powders, tablets, pills, capsules, lozenges, cachets, suppositories, and dispersible granules. A solid excipient can be one or more substances, which may also act as diluents, flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the excipient is preferably a finely divided solid, which is in a mixture with the finely divided inhibitor of the present invention. In tablets, the active ingredient is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. Suitable excipients are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or

cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

Liquid form composition include solutions, suspensions, and emulsions, for example, water, saline solutions, aqueous dextrose, glycerol solutions or water/propylene glycol solutions. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously or intranasally by a nebulizer. For parenteral injection, liquid preparations can be formulated in solution in, e.g. aqueous polyethylene glycol solution.

In a particularly preferred embodiment of this aspect, the pharmaceutical composition is in the form of a solution, suspension, or emulsion and is administered intranasally by a nebulizer.

Preferably, the pharmaceutical composition is in unit dosage form. In such form the composition may be subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged composition, the package containing discrete quantities of the composition, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, an injection vial, a tablet, a cachet, or a lozenge itself, or it can be the appropriate number of any of these in packaged form.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

Furthermore, such composition may also comprise other pharmacologically active substance such as but not limited to adjuvants and/or additional active ingredients.

Adjuvants in the context of the present invention include but are not limited to Examples of such adjuvants include but are not limited to inorganic adjuvants, organic adjuvants, oil-based adjuvants, cytokines, particulate adjuvants, virosomes, bacterial adjuvants, synthetic adjuvants, or synthetic polynucleotides adjuvants.

Additional active ingredients include but are not limited to other vaccine compounds or compositions. Preferably, the additional active ingredient is another viral vaccine, more preferably a vaccine against a DNA virus, a negative sense single stranded (ssRNA(-)) RNA virus or an ambisense RNA virus. Further preferred, the virus is selected from negative-single stranded (ssRNA(-)) RNA virus. Even more preferred, the virus is selected from enveloped ssRNA(-) viruses, more preferably from the group consisting of paramyxoviruses and orthomyxoviruses. Preferably, the additional active ingredient is a vaccine against paramyxoviruses, preferably selected from the group consisting of Pneumovirinae, Paramyxovirinae, Fer-de-Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, and Murayama-Virus. It is

particularly preferred that the Pneumovirinae is selected from the group consisting of Pneumovirus, (e.g. human respiratory syncytical virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV) and Metapneumovirus, (e.g. human metapneumovirus, avaian metapneumovirus). It is particularly preferred that the Paramyxovirinae is selected from the group consisting of Respirovirus (e.g. human parainfluenza virus 1 and 3), and Rubulavirus, (e.g. human parainfluenza virus 2 and 4). Alternatively or additionally, the additional active ingredient is preferably another viral vaccine against an orthomyxovirus, more preferably selected from the genus of Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotoviris and Isavirus. In even more preferred embodiments, the orthomxyovirus is Influenzavirus A, preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

In a fifth aspect the present invention provides for an expression system of the first aspect, the isolated protein mixture of the second aspect, the isolated host cell of the third aspect or the composition of the fourth aspect, for the use in the treatment or prevention of a viral disease.

In preferred embodiments of this aspect, the viral disease is caused by a DNA virus, a negative sense single stranded (ssRNA(-)) RNA virus or an ambisense RNA virus. Further preferred, the virus is selected from negative-single stranded (ssRNA(-)) RNA virus. Even more preferred, the virus is selected from enveloped ssRNA(-) viruses, more preferably from the group consisting of paramyxoviruses and orthomyxoviruses. The paramyxovirus is preferably selected from the group consisting of Pneumovirinae, Paramyxovirinae, Fer-de-Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, and Murayama-Virus. Even more preferably, the Pneumovirinae is selected from the group consisting of Pneumovirus, (e.g. human respiratory syncytical virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV) and Metapneumovirus, (e.g. human metapneumovirus, avaian metapneumovirus). Even more preferably, the Paramyxovirinae is selected from the group consisting of Respirovirus (e.g. human parainfluenza virus 1 and 3), and Rubulavirus, (e.g. human parainfluenza virus 2 and 4).Alternatively or additionally, the viral disease is preferably caused by an orthomyxovirus, more preferably selected from the genus of Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotoviris and Isavirus. In even more preferred embodiments, the orthomxyovirus is Influenzavirus A, preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

In a sixth aspect, the present invention provides for a method of treatment or prevention of a viral disease comprising the administration of effective amounts of the expression system of the first aspect, the isolated protein mixture of the second aspect, the isolated host cell of the third aspect or the composition of the fourth aspect for the use in the treatment or prevention of a viral disease.

In preferred embodiments of this aspect, the viral disease is caused by a DNA virus, a negative sense single stranded (ssRNA(-)) RNA virus or an ambisense RNA virus. Further preferred, the virus is selected from negative-single stranded (ssRNA(-)) RNA virus. Even more preferred, the virus is selected from enveloped ssRNA(-) viruses, more preferably from the group consisting of paramyxoviruses and orthomyxoviruses. The paramyxovirus is preferably selected from the group consisting of Pneumovirinae, Paramyxovirinae, Fer-de-Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, and Murayama-Virus. Even more preferably, the Pneumovirinae is selected from the group consisting of Pneumovirus, (e.g. human respiratory syncytical virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV) and Metapneumovirus, (e.g. human metapneumovirus, avaian metapneumovirus). Even more preferably, the Paramyxovirinae is selected from the group consisting of Respirovirus (e.g. human parainfluenza virus 1 and 3), and Rubulavirus, (e.g. human parainfluenza virus 2 and 4). Alternatively or additionally, the viral disease is preferably caused by an orthomyxovirus, more preferably selected from the genus of Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotoviris and Isavirus. In even more preferred embodiments, the orthomxyovirus is Influenzavirus A, preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

In a seventh aspect, the present invention provides for a method of enhancing an immune response against an immunogen comprising the administration of the expression system of the first aspect, the protein mixture of the second aspect, the cell of the third aspect and the composition of the fourth aspect.

In preferred embodiments of this aspect, the immunogen is a pathogen, more preferred the immunogen is a virus. Preferably, the virus is selected from the group consisting of a DNA virus, a negative sense single stranded (ssRNA(-)) RNA virus or an ambisense RNA virus. Further preferred, the virus is selected from negative-single stranded (ssRNA(-)) RNA virus. Even more preferred, the virus is selected from enveloped ssRNA(-) viruses, more preferably from the group consisting of paramyxoviruses and orthomyxoviruses. The paramyxovirus is preferably selected from the group consisting of Pneumovirinae, Paramyxovirinae, Fer-de-

Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, and Murayama-Virus. Even more preferably, the Pneumovirinae is selected from the group consisting of Pneumovirus, (e.g. human respiratory syncytical virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV) and Metapneumovirus, (e.g. human metapneumovirus, avaian metapneumovirus). Even more preferably, the Paramyxovirinae is selected from the group consisting of Respirovirus (e.g. human parainfluenza virus 1 and 3), and Rubulavirus, (e.g. human parainfluenza virus 2 and 4). Alternatively or additionally, the viral disease is preferably caused by an orthomyxovirus, more preferably selected from the genus of Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotoviris and Isavirus. In even more preferred embodiments, the orthomxyovirus is Influenzavirus A, preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

In an eighth aspect, the present invention provides nucleotide constructs encoding influenza hemagglutinin (HA), an expression system comprising these nucleotide constructs, and proteins or polyproteins encoded by the nucleotide constructs, wherein the HA0 cleavage site has a multibasic sequence.

The nucleic acid construct of this aspect comprises, essentially consists or consists of a polynucleotide encoding a modified influenza hemagglutinin (HA), wherein the HA0 cleavage site is modified by introducing one or more basic amino acids. Preferably, in the modified HA, the HA0 cleavage site of the consensus HA gene was substituted with the multibasic cleavage site of H5N1 that is cleaved by ubiquitous proteases to obtain a fully processed HA (H1p).

Influenza hemagglutinin (HA) is a protein belonging to the group of viral hemagglutinins found on the surface of the influenza viruses. It is an antigenic glycoprotein which is responsible for binding the virus to the cell that is being infected. HA proteins like influenza hemagglutinin bind to cells with sialic acid on the membranes, such as cells in the upper respiratory tract or erythrocytes. There are at least 16 different HA antigens. These serotypes or subtypes are named H1 through H16.

HA has two functions:

1. Recognition of target cells by binding to sialic acid-containing receptors.

2. Mediating the entry of the viral genome into the target cells by causing the fusion of host endosomal membrane with the viral membrane (envelop) In detail, HA binds to the monosaccharide sialic acid which is present on the surface of its target cells. The cell membrane then engulfs the virus and the portion of the membrane that encloses the virus forms an endosome. Then the endosome is acidified and being transformed it into a lysosome. As soon as

the pH within the endosome drops to about 6.0, the original folded structure of the HA molecule becomes unstable, causing it to partially unfold, releasing a hydrophobic portion of its peptide chain. This fusion peptide acts inserts into the endosomal membrane. Then, the rest of the HA molecule refolds into a new structure and causes the fusion of the viral membrane with the endosomal membrane such that the contents of the virus, including its RNA genome, are released into the cytoplasm of the cell.

To acquire its membrane fusion potential, HA0 must be cleaved into HA1 and HA2 by host cell proteases. Cleavage occurs at a linker sequence connecting the HA1 and HA2 subunits, which is located on a partially surface exposed loop. The H0 cleavage site of the influenza HA is located at about aa 340 in the H1N1 subtype (aa 339 to 344 of SEQ ID NO: 8), the H5N1 subtype (aa 337 to 346 of SEQ ID NO: 10) and H3N2 (aa 340 to 350 of SEQ ID NO: 20). The position of the H0 cleavage site in other subtypes of Influenza virus HA can be determined by the skilled person by conducting sequence alignments and analysing the homology of the sequences by methods well-known in this technical field. Influenza A virus HA of subtype H1N1 and H3N2 require cleavage by host cell proteases to transit into a fusion-competent state. Proteolytic activation of influenza viruses can occur in the Golgi apparatus or at the plasma membrane of infected cells, as well as in the extracellular space and in target cell vesicles, so the nature of the cleavage site and the respective activating proteases have important implications for the biological properties of influenza virus as well as for therapeutic intervention. HA of subtype H5N1 were shown to harbour several arginine and lysine residues at the cleavage site, with an R-X-R/K-R consensus sequence being indispensable for efficient cleavage. In addition, evidence was obtained that cleavage of HA might occur in the trans-Golgi network (TGN). It has been demonstrated that these viruses are activated by furin.

The amino acid sequence of SEQ ID NO: 8 is a consensus sequence derived from the alignment of 829 sequences of the H1N1 subtype annotated in the NCBI Influenza Virus Resource Database, circulating worldwide from April to September 2009. The amino acid sequence of SEQ ID NO: 9 is identical to SEQ ID NO: 8 with the exception that the natural H0 protease cleavage site has been substituted with a multibasic site derived from H5N1. The amino acid sequence of SEQ ID NO: 10 is a consensus derived from the alignment of 259 sequences of the H5N1 subtype annotated in the NCBI Influenza Virus Resource Database, infecting humans worldwide from 1990 to 2009. The amino acid sequence SEQ ID NO: 20 is the sequence of HA of influenza A virus subtype H3N2, strain A/Wellington/01/2004(H3N2). The amino acid sequence of SEQ ID NO: 21 is based on SEQ ID NO: 20 wherein the natural H0 protease cleavage site has been substituted with a multibasic site derived from H5N1

The amino acid sequence of SEQ ID NO: 11 is a NP consensus sequence which was designed on the basis of the alignment of the different influenza subtype consensus sequences. Further, the NP sequence of SEQ ID NO: 11 lacks the Nuclear Localization Signal residing in aa 6-8 (TRK to AAA) to increase cytoplasmic expression.

The amino acid sequence of SEQ ID NO: 12 is a M1 consensus sequence which was derived by alignment of different consensus sequences which were aligned and the most common amino acid at each position was chosen.

In a preferred embodiment of this aspect, the nucleic acid construct and/or the expression system comprising this nucleic acid construct, comprises elements to direct transcription and translation of the HA and the optional further proteins encoded by the nucleic acid construct and/or the expression system, which may be included in the preferred embodiments outlined below. Such elements included promoter and enhancer elements to direct transcription of mRNA in a cell-free or a cell-based based system, preferably a cell-based system. In another embodiment, wherein the polynucleotides are provided as translatable RNAs is envisioned that the expression system comprises those elements that are necessary for translation and/or stabilization of RNAs encoding the HA and/or the T cell inducing protein(s), e.g. polyA-tail, IRES, cap structures etc.

In a preferred embodiment of this aspect, the nucleic acid construct encodes a HA protein, peptide or variant thereof comprising a modified H0 cleavage site, wherein the HA is selected from the group of HA subtypes consisting of H1, H2, H3, H4, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16 or a variant thereof or a consensus sequence thereof, or a variant thereof or a consensus sequence of one or more of the HA subtypes selected from the group of HA subtypes consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16. Preferably, the HA subtypes are selected from the group consisting of H1, H2, H3, H7, H9, H10, or a variant thereof or a consensus sequence thereof, or a variant thereof or a consensus sequence of one or more of the HA subtypes selected from the group of HA subtypes consisting of H1, H2, H3, H5, H7, H9, H10. More preferred, the HA protein, peptide or variant thereof which comprises a modified H0 cleavage site, is a HA from subtype H1 or a variant thereof. Most preferred, the polynucleotide encoding the HA of the nucleotide construct has the sequence of SEQ ID NO: 9.

In a preferred embodiment of this aspect, the nucleic acid construct encodes a HA protein, peptide or variant thereof, wherein H0 cleavage site is modified by substituting at least one non-basic amino acid by a basic amino acid and/or by introducing at least one basic amino acid into the sequence of the H0 cleavage site. Preferably, the basic amino acid is selected from the group consisting of arginine (Arg; R), lysine (Lys; K) and histidine (His, H). More

preferably, the basic amino acid is selected from the group consisting of arginine (Arg; R) and lysine (Lys; K).

Preferably, the cleavage site comprises a sequence of 6 to 12 amino acids, more preferably 10-12 amino-acids.

Preferably, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% to the amino acids of the polypeptide forming the H0 cleavage site are basic amino acids.

Preferably, the HA0 cleavage site of has a sequence selected from the group consisting of PQRERRRKKR (SEQ ID NO: 15), PQRESRRKKR (SEQ ID NO: 16), PQGERRRKKR (SEQ ID NO: 17), PLRERRRKR (SEQ ID NO: 18) and PQRETR (SEQ ID NO: 19). Most preferred, the HA0 cleavage site of has the sequence PQRERRRKKR (SEQ ID NO: 15).

In Table 1 the sequences of the H0 cleavage site of H1N1 strain and the sequences of the H0 cleavage site of H5N1 strain are compared in their respective context of the HA amino acid chain. In the first line, the consensus sequence of the H0 polybasic cleavage site of the highly pathogenic H5N1 subtype is indicated. In the second line, the amino acid sequence of the H0 cleavage site of the HA of the H1N1 wild-type strain is indicated. In the third line the engineered sequence of the polybasic cleavage site in the H1p protein, replacing the natural sequence.

Tab. 1

H5N1 cleavage site	LATGLRNS **PQRERRRKKR** GLFGAIA.....
H1N1 cleavage site	LATGLRNV **PSIQSR** GLFGAIA.....
H1p cleavage site	LATGLRNI **PQRERRRKKR** GLFGAIA.....

In embodiments of the eighth aspect of the present invention, the nucleic acid construct is part of an expression system encoding the modified HA and a second polynucleotide. In this expression system the polynucleotide encoding the modified HA and the second polynucleotide are comprised on separate vectors or on the same vector. Accordingly, the polynucleotide encoding the modified HA may be comprised on one vector and the second polynucleotide may be comprised on a second vector. Alternatively or additionally, the polynucleotide encoding the modified HA and the second polynucleotide may be comprised on the same vector. It is preferred that the polynucleotide encoding the modified HA and the second polynucleotide are comprised on the same vector. It is particularly preferred that the polynucleotide encoding the modified HA and the second polynucleotide comprised on the same vector are linked in such that they are expressed as a polyprotein. Preferably, the polynucleotide encoding the modified HA and the second polynucleotide form an open reading frame.

It is preferred that the polynucleotide encoding the modified HA and the second polynucleotide are expressed as an artificial polyprotein. In the context of the present invention the term "artificial polyprotein" is directed at polyproteins which are not naturally occurring, e.g. which are generated by using recombinant DNA techniques. Accordingly, the proteins, peptides or variants thereof encoded in this artificial polyprotein are preferably derived from pathogens which genome do not encode a polyprotein comprising the proteins, peptides or variants encoded by the polynucleotide encoding the modified HA and second polynucleotide of the invention. Preferably, the polynucleotide encoding the modified HA and the second polynucleotide are both derived from influenza A viruses.

In preferred embodiments of the eighth aspect, the second polynucleotide encodes a protein or variant thereof, which induces a T cell response, and which is, preferably, a non-structural and/or internal protein of influenza A virus. Preferably, the non-structural and/or internal protein encoded by the second polynucleotide is selected from the group consisting of NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2).

It is preferred that the amino acid sequence of the modified HA and/or the non-structural (internal) protein encoded by the second polynucleotide comprises consecutive segments or a consensus sequence of one or more different virus isolates.

In the context of the present invention it is preferred that the term "segment" refers to a part of a protein or polyprotein. It is particularly preferred that such segment folds and/or functions independently of the rest of the protein or polyprotein such as but not limited to a domain, an epitope or a fragment thereof. It is understood that a protein variant in the context of the present invention differs in comparison to its parent polypeptide in changes in the amino acid sequence such as amino acid exchanges, insertions, deletions, N-terminal truncations, or C-terminal truncations, or any combination of these changes, which may occur at one or several sites whereby the variant exhibits at least 80% sequence identity to its parent polypeptide.

In a further preferred embodiment, a membrane attachment domain of the modified HA or a variant thereof is functionally deleted, thus, either being structurally deleted or structurally present but not fulfilling its biological function. In a particularly preferred embodiment, the amino acid sequence corresponding to the membrane attachment domain is deleted. The deletion of the membrane attachment region serves the purpose of ascertaining that the anti-pathogenic B cell response inducing protein is secreted from the cell into which the expression system of the invention has been introduced.

In a further preferred embodiment of this apect the modified HA comprises a secretion signal, which targets the protein to the endoplasmatic reticulum (ER). Such secretion signals are present preferably in the context of a deleted membrane attachment domain. The skilled person

is well aware of various such secretion signals, which may be used as heterologous secretion signals, e.g. added to the N-terminus of the modified HA. Alternatively or additionally a naturally occurring secretion signal may be used, which is, e.g., present in the majority of structural and/or surface viral proteins. Thus, if naturally present in the respective protein, it is preferred that the secretion signal is maintained in a modified version of the structural and/or surface protein.

In embodiments of the eighth aspect, the non-structural protein is a conserved internal protein suitable for inducing a T cell mediated immune response against the pathogen involving the activation of antigen-specific T lymphocyte such as but not limited to cytotoxic T cells (CTLs), T helper cells (T_H cells), central memory T cells (TCM cells), effector memory T cells (TEM cells), and regulatory T cells (Treg cells). Thus, preferably the T cell inducing protein of the pathogen does not comprise a secretion signal.

In the context of the present invention, the modified HA or variant thereof is located either N- or C-terminally with respect to the protein, peptide or variant thereof encoded by the second polynucleotide. In a preferred embodiment, the protein, peptide or variant thereof encoded by the second polynucleotide is located N-terminally with respect to the modified HA or variant thereof.

In preferred embodiments of the eighth aspect, a polynucleotide encoding a cleavage site is positioned between the modified HA or variant thereof and the second polynucleotide. It is within the scope of the present invention that that any two proteins can or cannot be connected or linked by a cleavage site.

It is preferred that this cleavage site is either a self-cleaving site (i.e. a cleavage site within the amino acid sequence where this sequence is cleaved or is cleavable without such cleavage involving any additional molecule or where the peptide-bond formation in this sequence is prevented in the first place) or an endopeptidase cleavage site (i.e. a cleavage cite within the amino acid sequence where this sequence is cleaved or is cleavable by an endopeptidase, e.g. trypsin, pepsin, elastase, thrombin, collagenase, furin, thermolysin, endopeptidase V8, cathepsins). More preferably, the self-cleaving site is a 2A cleavage site selected from the group consisting of a viral 2A peptide or 2A-like peptide of Picornavirus, insect viruses, Aphtoviridae, Rotaviruses and Trypanosoma, preferably wherein the 2A cleavage site is the 2 A peptide of foot and mouth disease virus. Alternatively or additionally, the polyprotein of the present invention can be cleaved by an autoprotease, i.e. a protease which cleaves peptide bonds in the same protein molecule which also comprises the protease. Examples of such autoproteases are the NS2 protease from flaviviruses or the VP4 protease of birnaviruses

In the context of the present invention, the cleavage site can be positioned N-terminally with respect to the modified HA or variant thereof and C-terminally with respect to the protein, peptide or variant thereof encoded by the second polynucleotide. Alternatively the cleavage site can be positioned C-terminally with respect to the modified HA or variant thereof and N-terminally with respect to the protein, peptide or variant thereof encoded by the second polynucleotide.

In preferred embodiment of the eighth aspect, the expression system further comprises a third polynucleotide encoding a protein, peptide or a variant thereof of a pathogen.

It is preferred that the protein, peptide or variant thereof encoded by the third polynucleotide differs from the modified HA or variant thereof or from the protein, peptide or variant thereof encoded by the second polynucleotide. Preferably, the proteins, peptides or variants thereof encoded by the first, second and the third polynucleotide differ from each other in that they comprise amino acid sequences of different proteins.

In preferred embodiments of the eighth aspect, the third polynucleotide encodes a protein or variant thereof, which induces a T cell response, and which is, preferably, a non-structural and/or internal protein of influenza A virus. Preferably, the non-structural and/or internal protein encoded by the third polynucleotide is selected from the group consisting of NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 or PB1-F2 (PB1F2).

In preferred embodiments a polynucleotide encoding a linker is positioned between the second polynucleotide and the third polynucleotide. It is preferred that the linker is a flexible linker, preferably a flexible linker comprising an amino acid sequence according to SEQ ID NO: 6 (Gly-Gly-Gly-Ser-Gly-Gly-Gly).

In preferred embodiments the third polynucleotide is comprised on a separate or on the same vector as the polynucleotide encoding the modified HA or variant thereof and/or the second polynucleotide.

Accordingly, the polynucleotide encoding the modified HA or variant thereof is comprised on one vector and the second polynucleotide is comprised on a second vector and the third polynucleotide is comprised on a third vector. Alternatively or additionally, the polynucleotide encoding the modified HA or variant thereof and the second polynucleotide are comprised on the same vector and the third polynucleotide is comprised on a separate vector, or the polynucleotide encoding the modified HA or variant thereof and the third polynucleotide are comprised on the same vector and the second polynucleotide is comprised on a separate vector, or the second and the third polynucleotide are comprised on the same vector and the polynucleotide encoding the modified HA or variant thereof is comprised on a separate vector. Alternatively or additionally, the polynucleotide encoding the modified HA or variant thereof

and the second and the third polynucleotide are comprised on the same vector. It is preferred that the polynucleotide encoding the modified HA or variant thereof and the second and the third polynucleotide may be comprised on the same vector. It is particularly preferred that the polynucleotide encoding the modified HA or variant thereof and the second and the third polynucleotide comprised on the same vector are linked in such that they are expressed as a polyprotein. Preferably, the polynucleotide encoding the modified HA or variant thereof and the second and the third polynucleotide comprised on the same vector form an open reading frame.

In preferred embodiments of this aspect, the vector or vectors comprising the polynucleotide encoding the modified HA or variant thereof, and the second and/or the third polynucleotide is/are selected from the group consisting of plasmid, cosmid, phage, virus, and artificial chromosome. More preferably, a vector suitable for practicing the present invention is selected from the group consisting of plasmid vectors, cosmid vectors, phage vectors, preferably lambda phage and filamentous phage vectors, viral vectors, adenovirus vectors (e.g., non-replicating Ad5, Ad11, Ad26, Ad35, Ad49, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd 73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, and PanAd3 vectors or replication-competent Ad4 and Ad7 vectors), adeno-associated virus (AAV) vectors (e.g., AAV type 5 and type 2), alphavirus vectors (e.g., Venezuelan equine encephalitis virus (VEE), sindbis virus (SIN), semliki forest virus (SFV), and VEE-SIN chimeras), herpes virus vectors (e.g. vectors derived from cytomegaloviruses, like rhesus cytomegalovirus (RhCMV) (14)), arena virus vectors (e.g. lymphocytic choriomeningitis virus (LCMV) vectors (15)), measles virus vectors, pox virus vectors (e.g., vaccinia virus, modified vaccinia virus Ankara (MVA), NYVAC (derived from the Copenhagen strain of vaccinia), and avipox vectors: canarypox (ALVAC) and fowlpox (FPV) vectors), vesicular stomatitis virus vectors, retrovirus, lentivirus, viral like particles, and bacterial spores. The vectors ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63 and ChAd82 are described in detail in WO 2005/071093. The vectors PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146, and ChAd147 are described in detail in WO 2010/086189. It is particularly preferred that the vector is selected from the group consisting of MVA, ChAd63 and PanAd3.

In preferred embodiments of this aspect, the nucleotide construct or the expression system or the vector or vectors comprising the polynucleotide of the nucleotide construct or the expression system may encompass “expression control sequences” that regulate the expression of the gene of interest. Typically, expression control sequences are polypeptides or

polynucleotides such as but not limited to promoters, enhancers, silencers, insulators, or repressors.

In a particularly preferred embodiment of this aspect, the expression system is defined according to the embodiments of the first aspect of the present invention directed at expressing systems comprising polynucleotides encoding proteins, peptides or variants thereof from orthomyxovirus, preferably proteins, peptides or variants from influenza A viruses.

In preferred embodiments, the nucleic acid construct and/or the expression system of the eighth aspect is for use in medicine. In more preferred embodiments, the nucleic acid constructs, the expression systems or the proteins of this aspect are for use in the prophylaxis or treatment of an influenza A virus infection and/or in the manufacturing of medicament for use in the prophylaxis or treatment of an influenza A virus infection and/or for use in methods of prophylaxis or treatment of an influenza A virus infection.

In preferred embodiments the expression system is for use in enhancing an immune response. In more preferred embodiments, the expression system is for use in enhancing an anti-pathogenic B cell immune response against an influenza A virus infection, more preferably an influenza A virus as defined in the first aspect of the invention.

In a ninth aspect, the present invention provides the use of the multibasic HA0 cleavage site as defined in the eighth aspect for constructing a nucleic acid construct or an expression systems capable of expressing the modified influenza hemagglutinin (HA) of the eighth aspect *in vitro* and/or *in vivo*. Furthermore, this aspect provides the isolated protein mixture, the protein and/or polyprotein encoded by the nucleic acid construct or expression system constructed according to this aspect.

In a tenth aspect, the invention provides an isolated protein mixture encoded by the expression system of the eighth aspect. Preferably, the isolated protein mixture contains, essentially contains or comprises one or more of the proteins or polyproteins encoded by the nucleic acid construct or the expression system of the eighth aspect. In preferred embodiments, the isolated protein mixture is for use in medicine. In particularly preferred embodiments, the isolated protein mixture is for use in the prophylaxis or treatment of a viral infection, particularly preferably for use in the prophylaxis or treatment of an influenza A virus infection and/or in the manufacturing of medicament for use in the prophylaxis or treatment of an influenza A virus infection and/or for use in methods of prophylaxis or treatment of an influenza A virus infection..

In an eleventh aspect, the invention provides an isolated host cell containing the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect and/or the protein mixture of the tenth aspect. It is understood that such host cell includes but is not limited to prokaryotic (e.g. a bacterial cell) or eukaryotic cells (e.g. a fungal, plant or animal

cell). In preferred embodiments of this aspect, the host cell is for use in medicine. In particularly preferred embodiments, the host cell is for use in the prophylaxis or treatment of an influenza A virus infection and/or in the manufacturing of medicament for use in the prophylaxis or treatment of an influenza A virus infection and/or for use in methods of prophylaxis or treatment of an influenza A virus infection.

In a twelfth aspect, the present invention provides a composition comprising the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect, or the protein mixture of the tenth aspect, and a pharmaceutical acceptable carrier and/or excipient. Preferably, such composition is a pharmaceutical composition.

The composition of the twelfth aspect contains a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

The compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

For preparing pharmaceutical compositions of the present invention, pharmaceutically acceptable carriers can be either solid or liquid.

Solid form compositions include powders, tablets, pills, capsules, lozenges, cachets, suppositories, and dispersible granules. A solid excipient can be one or more substances, which may also act as diluents, flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the excipient is preferably a finely divided solid, which is in a mixture with the finely divided inhibitor of the present invention. In tablets, the active ingredient is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. Suitable excipients are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

Liquid form composition include solutions, suspensions, and emulsions, for example, water, saline solutions, aqueous dextrose, glycerol solutions or water/propylene glycol solutions. A saline solution is a preferred carrier when the pharmaceutical composition is administered

intravenously or intranasally by a nebulizer. For parenteral injection, liquid preparations can be formulated in solution in, e.g. aqueous polyethylene glycol solution.

In a particularly preferred embodiment of this aspect, the pharmaceutical composition is in the form of a solution, suspension, or emulsion and is administered intranasally by a nebulizer.

Preferably, the pharmaceutical composition is in unit dosage form. In such form the composition may be subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged composition, the package containing discrete quantities of the composition, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, an injection vial, a tablet, a cachet, or a lozenge itself, or it can be the appropriate number of any of these in packaged form.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

Furthermore, such composition may also comprise other pharmacologically active substance such as but not limited to adjuvants and/or additional active ingredients.

Adjuvants in the context of the present invention include but are not limited to Examples of such adjuvants include but are not limited to inorganic adjuvants, organic adjuvants, oil-based adjuvants, cytokines, particulate adjuvants, virosomes, bacterial adjuvants, synthetic adjuvants, or synthetic polynucleotides adjuvants.

Additional active ingredients include but are not limited to other vaccine compounds or compositions. Preferably, the additional active ingredient is another viral vaccine, more preferably a vaccine against a DNA virus, a negative sense single stranded (ssRNA(-)) RNA virus or an ambisense RNA virus. Further preferred, the virus is selected from negative-single stranded (ssRNA(-)) RNA virus. Even more preferred, the virus is selected from enveloped ssRNA(-) viruses, more preferably from the group consisting of paramyxoviruses and orthomyxoviruses. Preferably, the additional active ingredient is a vaccine against paramyxoviruses, preferably selected from the group consisting of Pneumovirinae, Paramyxovirinae, Fer-de-Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, and Murayama-Virus. It is particularly preferred that the Pneumovirinae is selected from the group consisting of Pneumovirus, (e.g. human respiratory syncytical virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV) and Metapneumovirus, (e.g. human metapneumovirus, avaian metapneumovirus). It is particularly preferred that the Paramyxovirinae is selected from the group consisting of Respirovirus (e.g. human parainfluenza virus 1 and 3), and Rubulavirus, (e.g. human parainfluenza virus 2 and 4). Alternatively or additionally, the additional active ingredient is preferably another viral vaccine against an orthomyxovirus, more preferably

selected from the genus of Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotoviris and Isavirus. In even more preferred embodiments, the orthomxyovirus is Influenzavirus A, preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

In a thirteenth aspect, the present invention provides the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect, the protein mixture of the tenth aspect, the cell of the eleventh aspect and the composition of the twelfth aspect, for the use in medicine in particular in the treatment or prevention of influenza A virus infections. The influenza A virus is preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

In a fourteenth aspect, the present invention provides for a method of treatment or prevention of an influenza A virus infections comprising the administration of an effective amount of the nucleotide constructs, the expression system or the proteins or polyproteins of the eighth aspect, the protein mixture of the tenth aspect, the cell of the eleventh aspect and the composition of the twelfth aspect. The influenza A virus is preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

In a fifteenth aspect, the present invention provides for a method of enhancing an immune response comprising the administration of the nucleotide constructs or the expression system or the proteins or polyproteins of the eighth aspect, the protein mixture of the tenth aspect, the cell of the eleventh aspect and the composition of the twelfth aspect. In a preferred embodiment of this aspect, the method enhances an immune response against influenza A virus. The influenza A virus is preferably selected from the influenza A virus subtypes H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, more preferably the influenza A virus subtype H1N1.

The following examples are merely illustrative of the present invention and should not be construed to limit the scope of the invention as indicated by the appended claims in any way.

Examples

Example 1: Design and synthesis of DNA encoding optimised RSV antigen

Consensus vaccine

Several computational alternatives to isolate-based vaccine design exist. One approach is reconstruction of the most recent common ancestor (MRCA) sequence (9). In this type of analysis, the ancestral state is an estimate of the actual sequence that existed in the past (i.e., it comes directly from the reconstructed history). Another type of computational analysis is a center of the tree (COT) approach. The COT approach identifies a point on the unrooted phylogeny, where the average evolutionary distance from that point to each tip on the phylogeny is minimized. Advocates of this approach state that because the COT is a point on the phylogeny, the estimated COT sequence will have the same advantages as the estimated ancestral sequence. See, for example, U.S. Application 2005/0137387 A1. However, this COT approach is sufficiently complex that reducing it to practice for a large and heterologous data set such as the Influenza sequence database is not practical with technology. Overall, the MRCA and COT approaches are impractical for application to the complex Influenza sequence database.

A third type of computational analysis is the consensus sequence approach. Because the consensus sequence is composed of the amino acid most commonly observed at each position, it likely represents the most-fit state of the virus. Thus, effective evasion of the immune response by selection of a sequence divergent from consensus may result in a less fit virus from a replicative standpoint. The consensus sequence approach favors heavily sampled sublineages and deemphasizes outliers. As such, the approaches utilized herein are far more straightforward than the other types of computational analyses. Furthermore, these approaches can use the entire data set for RSV. One advantage of the consensus sequence is that it minimizes the genetic differences between vaccine strains and contemporary isolates, effectively reducing the extent of diversity by half, and thus it may have enhanced potential for eliciting cross-reactive responses.

Vaccine design

To design the vaccine antigen of the present invention, protein sequences of the F0-, N-, and M2-1- proteins of RSV were retrieved from the National Center for Biotechnology Information (NCBI) RSV Resource database (http://www.ncbi.nlm.nih.gov). Protein sequences were chosen from different RSV subtype A strains.

A F0 consensus sequence was derived by alignment of all non-identical sequences of the F-protein using MUSCLE version 3.6 and applying the majority rule. The vaccine's F0 consensus sequence was designed on the basis of the alignment of the different RSV sequences. The sequence similarity of the vaccine consensus F0 sequence was measured performing BLAST analysis, which stands for Basic Local Alignment Search Tool and is publicly available through the NCBI. The highest average similarity of the consensus sequence, calculated

compared to all RSV sequences in the database, was 100 % with respect to the human respiratory syncytial virus A2 strain.

Further, the vaccine's F0 sequence lacks the transmembrane region residing in amino acids 525 to 574 to allow for the secretion of F0ΔTM.

Finally, the vaccine F0ΔTM sequence was codon-optimized for expression in eukaryotic cells.

The vaccine's N consensus sequence was derived by alignment of all non-identical sequences of the N-protein using MUSCLE version 3.6 and applying the majority rule. BLAST analysis of the N consensus sequence found the best alignment with the human respiratory syncytial virus A2 strain. The vaccine's N sequence was then codon-optimized for expression in eukaryotic cells.

A M2-1 consensus sequence was derived by alignment of all non-identical sequences of the M2-1-protein using MUSCLE version 3.6 and applying the majority rule. BLAST analysis of the M2-1 consensus sequence found the best alignment with the human respiratory syncytial virus A2 strain. Finally, the vaccine M2-1 sequence was codon-optimized for expression in eukaryotic cells.

The vaccines F0ΔTM sequence and N sequence were spaced by the cleavage sequence 2A of the Foot and Mouth Disease virus. The vaccines N sequence and M2-1 sequence were separated by a flexible linker (GGGSGGG; SEQ ID NO: 6).

Finally, the codon-optimized viral genes were cloned as the single open reading frame F0ΔTM-N-M2-1. A schematic diagram of the antigen composition is given in Fig. 1.

Generation of DNA plasmids encoding F0ΔTM and F0ΔTM-N-M2-1

Consensus F0ΔTM, N and M2-1 sequences were optimized for mammalian expression, including the addition of a Kozak sequence and codon optimization. The DNA sequence encoding the multi-antigen vaccine was chemically synthesized and then sub-cloned by suitable restriction enzymes EcoRV and NotI into the pVJTetOCMV shuttle vector under the control of the CMV promoter.

Generation of PanAd3 viral-vectored RSV vaccine

A viral-vectored RSV vaccine PanAd3/F0ΔTM-N-M2-1 was generated which contains a 809 aa polyprotein coding for the consensus F0ΔTM, N and M2-1 proteins fused by a flexible linker.

Bonobo Adenovirus type 3 (PanAd3) is a novel adenovirus strain with improved seroprevalence and has been described previously.

Cloning of F0ΔTM-N-M2-1 from the plasmid vector pVJTetOCMV/F0ΔTM-N-M2-1 into the PanAd3 pre-Adeno vector was performed by cutting out the antigen sequences flanked by homologous regions and enzymatic *in vitro* recombination.

Analysis of antigen expression in mammalian cells

To control that the unique combination of viral antigens was efficiently expressed and correctly processed into mammalian cells, Hela cells were transfected with 10 µg of DNA plasmid encoding the F0ΔTM-N-M2-1 antigen. Cells were cultured for 36 hours before the supernatant was collected and cell lysates were prepared. Proteins were separated by SDS-PAGE and blotted onto nylon filters. A mouse monoclonal antibody (mAb8) raised against the M viral protein (gift from Dr. Geraldine Taylor) was used to reveal the expressed proteins.

As shown in Fig. 2 and 3, the fused viral protein N-M2-1 is very efficiently released from the polyprotein by the 2A cleavage site and recognized as a major band by mAb8. Very few high molecular weight precursor is present at steady-state in the cells. Lysates of Hep2-cells infected with RSV strain A were used as control.

Non-Reducing SDS-PAGE and Western blot analysis of the cell culture medium showed that the F-protein deleted of the trans-membrane region is secreted into the supernatant (see Fig. 3, lane RSV). The molecular weight of the F-protein in the supernatant is consistent with homotrimeric F-protein, which is its native configuration.

Example 2: Vaccine immunogenicity in mice

Anti-F antibodies by DNA immunization

DNA plasmids encoding F0ΔTM-N-M2-1 or F0ΔTM alone were used to immunize mice by DNA plasmid injection and electroporation (GET) with a regimen of priming and boosting at three weeks post prime. Sera of immunized mice were collected two weeks after boosting and pooled.

Supernatants from Hela cells infected with PanAd3/F0ΔTM-N-M2-1 at MOI 250 were separated on non-reducing SDS-PAGE, blotted onto nylon filters and probed with different dilutions of sera from mice immunized with F0ΔTM or F0ΔTM-N-M2-1.

As shown in Fig. 4A and B, the antibody titers raised by the F-protein expressed in the context of the vaccine antigen are at least 30 times higher than those elicited by the F-protein alone. Thus, the F0ΔTM-N-M2-1 antigen has superior immunogenic properties in inducing B-cell responses in mice.

T cell response

The immunological potency of the chimpanzee adenoviral vector PanAd3 bearing the RSV vaccine antigen F0ΔTM-N-M2-1 was evaluated in mice.

Groups of Balb/C mice were immunized by intramuscular injection in the quadriceps with increasing dose of PanAd3/ F0ΔTM-N-M2-1. 4 weeks after vaccination mice were sacrificed and splenocytes were subjected to IFNγ-Elispot assay using mapped immunodominant peptides from RSV F- and M-proteins (peptide GWYTSVITIELSNIKE (F aa 51-66) peptide KYKNAVTEL (F aa 85-93) and peptide SYIGSINNI (M aa 282-290)).

As shown in Fig. 5, a potent T cell response was observed against known Balb/C immunodominant epitopes against RSV F and M proteins.

Example 3: Induction of neutralizing antibodies by H1p

The novel chimeric H1p protein engineered to contain the multibasic HA0 cleavage site from H5N1 is efficiently expressed and fully cleaved in transfected HeLa cells. The equivalent protein with the wild type cleavage site, H1, is not cleaved in HeLa cells, as shown in Fig. 7.

In order to control that the chimeric H1p protein is correctly displayed on the cell membrane, a whole-cell FACS binding assay has been performed using a polyclonal anti-HA serum to reveal the transfected protein on the cell surface. As shown in Fig.8, H1p is exposed on the cell membrane as efficiently as the corresponding wild type HA protein.

To measure the immunological potency of H1p, Balb/C mice were immunized with plasmid DNA vectors encoding the modified H1p and the unmodified H1 (PVJ-H1p and PVJ-H1, respectively). The sera from immunized animals have been analyzed by ELISA on purified recombinant HA protein (H1N1California2009). The anti-HA titers elicited by the engineered H1p protein were surprisingly higher than those elicited by the HA bearing the wild type protease cleavage site (Fig. 9).

To confirm and expand these results, the sera from H1 and H1p immunized animals were tested for their capacity to neutralize the infection of retroviral vectors pseudotyped with the Flu HA protein in a cell culture based assay. Pseudovirions are infectious for a single cycle of infection in which they express the reporter gene luciferase. Fig. 10 shows the serum neutralization capacity in a HA (H1N1Mexico2009) pseudotyped virus particles infection assay on MDCK cells. The result confirms that the antibodies elicited by H1p have greater neutralizing activity than those induced by H1 protein.

Example 4: Enhanced antibody titer by a polyprotein comprising NP, M1 and H1p

Head to head comparison of the immunological potency of the H1p and NPM1H1p revealed that the HA protein expressed in the context of the triple antigen induces higher antibody titer than HA alone. Fig. 11 shows the results of an ELISA assay where a recombinant HA (H1N1California 2009) was coated on the bottom of 96 well plate. Serial dilutions of sera of animals immunized with H1p and NPM1H1p were put on the plate and the bound IgG were revealed with an anti-mouse IgG secondary antibody. As already observed for the RSV antigen, the co-expression of internal antigens (NP and M1) with the surface exposed antigen (HA) improves the humoral response directed to the latter protein.

Example 5: Processing of the novel FLU antigen composed of NP, M1 and H1p

To control the expression and processing of the vaccine antigen proteins, HeLa cells have been transfected with an expression plasmid containing NPM1H1p under the control of the CMV promoter. Cells were harvested 48 hours after transfection. Half cells were lysed for Western Blot analysis (Fig.12) and half were incubated with a commercially available antibody C179 (Okuno Y, JVI 1994), which binds the stem region of the HA protein (Fig.13) and analysed by FACS. Western blot analysis of the total cell lysate shows a unique 70 kDa band which correspond to the NPM1 fusion protein (Fig. 12). This indicates that the antigen is fully and correctly processed out of the 2A cleavage site. Fig. 13 shows that the released H1p protein is then displayed on the cell membrane and correctly folded, as detected by the use of a conformation-dependent antibody C179 which binds to the HA stem region. Accordingly, the novel FLU antigen composed of NP, M1 and H1p is correctly processed and the released HA protein is displayed on the cell surface and recognized by a conformational antibody.

References

1) Ono & Freed, (2005), Adv. Virus Res., 273:5419-5442
2) Collins P et al., (1996). Parainfluenza viruses, 1205-1241. In Fields et al. (ed.), Fields Virology. Lippincot-Raven Publishers, Philadelphia (PA) USA
3) Shay DK et al., (1999), Bronchiolitis-Associated Hospitalizations Among US Children, 1980-1996. JAMA 282:1440-1446
4) Simoes EA & Carbonell-Estrany X (2003), Pediatr Infect Dis J 22:S13-8; discussion S18-20.
5) Collins PL & Graham BS (2008), J Virol 82:2040-55
6) Falsey AR et al., (2005), N Engl J Med 352:1749-59

7) Fleming DM & Elliot AJ (2007), Eur Respir J 30:1029-31

8) Cardenas S et al., (2005), Expert Rev Anti Infect Ther 3:719-26

9) Kim HW et al., (1969), Amer J Epidemiol 89:422-434

10) Delgado MF et al., (2009), Nat Med 15:34-41

11) Graham BS et al., (1993), J Immunol 151:2032-2040

12) Polack FP et al., (2002), J Exp Med 196:859-865

13) Castilow EM & Varga SM, (2008), Future Virol 3:445-454

14) Hansen S et al., (2011), Nature 473:523-527

15) Flatz, L et al. (2010), Nature Med 16:339-345

Sequence Listing – Free Text Information

SEQ ID NO: 1 F protein minimal sequence

SEQ ID NO: 2 F0ΔTM

SEQ ID NO: 3 N protein minimal sequence

SEQ ID NO: 4 N protein of RSV

SEQ ID NO: 5 M2-1 protein of RSV

SEQ ID NO: 6 peptide linker

SEQ ID NO: 7 F0ΔTM-N-M2-1

SEQ ID NO: 8 HA subtype H1 consensus sequence

SEQ ID NO: 9 HA subtype H1 consensus sequence modified H0 cleavage site

SEQ ID NO: 10 HA subtype H5 consensus sequence

SEQ ID NO: 11 NP consensus sequence

SEQ ID NO: 12 M1 consensus sequence

SEQ ID NO: 13 NP-M1-HA (amino acid sequence)

SEQ ID NO: 14 NP-M1-HA (nucleic acid sequence)

SEQ ID NO: 15 H0 cleavage site sequence

SEQ ID NO: 16 H0 cleavage site sequence

SEQ ID NO: 17 H0 cleavage site sequence

SEQ ID NO: 18 H0 cleavage site sequence

SEQ ID NO: 19 H0 cleavage site sequence

SEQ ID NO: 20 HA subtype H3 strain A/Wellington/01/2004(H3N2)

SEQ ID NO: 21 HA subtype H3 strain A/Wellington/01/2004(H3N2) modified H0 cleavage site

CLAIMS

1. A composition comprising an expression system comprising an adenovirus viral vector comprising polynucleotides encoding pneumovirus proteins, and a pharmaceutically acceptable carrier and/or excipient, wherein the expression system comprises a first polynucleotide encoding at least one protein, peptide or variant thereof, which induces a T cell response, a second polynucleotide encoding at least one protein, peptide or variant thereof, which induces an anti-pathogenic B cell response, and a third polynucleotide encoding a protein, peptide or variant thereof, which induces a T cell response, wherein:

   the pneumovirus is human respiratory syncytical virus (RSV);

   the protein, peptide or variant thereof which induces a T cell response, encoded by the first polynucleotide, is a non-structural and/or internal protein of RSV selected from the group consisting of Matrix proteins M and M2;

   the protein, peptide or variant thereof which induces an anti-pathogenic B cell response, encoded by the second polynucleotide, is a structural and/or surface protein of RSV which is the fusion protein (F), and;

   the protein, peptide or variant thereof which induces a T cell response, encoded by the third polynucleotide, is a non-structural and/or internal protein of RSV which is the nucleoprotein N; and

   the first polynucleotide, the second polynucleotide and the third polynucleotide are linked such that they are expressed as an artificial polyprotein.

2. The composition of claim 1, wherein a polynucleotide which encodes a cleavage site is positioned between the first polynucleotide and the second polynucleotide.

3. The composition of claim 2, wherein the cleavage site is a self-cleaving site or an endopeptidase cleavage site, wherein the self-cleaving site is selected from the group consisting of a viral 2A peptide or 2A-like peptide of Picornavirus, insect viruses, Aphtoviridae, Rotaviruses and Trypanosoma.

4. The composition of claim 3 wherein the 2A cleavage site is the 2A peptide of foot and mouth disease virus.

5. The composition of any of claims 1 to 4, wherein a polynucleotide encoding a linker, such as a flexible linker, is positioned between the second polynucleotide and the third polynucleotide.

6. The composition of any of claims 1 to 5, wherein the viral vector is selected from the group consisting of PanAd1, PanAd2, PanAd3, ChAd55, ChAd 73, ChAd83, ChAd146, ChAd147, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63 and ChAd82.

7. The composition of any of claims 1 to 6, comprising a further active ingredient, preferably selected from the group consisting of an adjuvant and an active ingredient.

8. The composition according to any one of claims 1 to 7, for use in the treatment or prevention of a disease caused by RSV infection.

9. The use of the composition according to any one of claims 1 to 7 in the manufacture of a medicament for the treatment or prevention of a disease caused by RSV infection.