TITLE

LASSA VACCINE

FIELD OF THE INVENTION

The application generally relates to recombinant genetic constructs comprising the recombinant measles virus and expressing at least one Lassa virus polypeptide, protein, antigen, or antigenic fragment thereof. The application also relates to the uses of genetic constructs or viruses, and more particularly their applications for inducing protection against the Lassa virus (LASV), and/or the measles virus (MV or MeV).

The means of the invention are more particularly dedicated to a recombinant nucleic acid construct allowing the expression of at least one of the following polypeptides of the LASV, or a truncated version thereof or an antigenic fragment thereof: the Nucleoprotein (NP), the Glycoprotein precursor (GPC), the zinc-binding protein (Z), or a mutated version of the native NP protein (mutated NP or mNP) wherein the exonuclease activity of the NP protein has been knocked down.

The invention also relates to a recombinant MeV-LASV virus expressing at least one of the previously mentioned LASV polypeptide, antigenic fragment thereof or antigen thereof or a truncated version thereof, namely NP, mNP, GPC and/or Z. The invention also concerns immunogenic particles expressed by the measles virus and comprising a LASV polypeptide, in particular at least the GPC polypeptide, or protein, or antigenic fragment thereof and/or infectious Virus-like particles (VLPs) that contains at least the Z polypeptide, or protein, or antigenic fragment thereof, said immunogenic particles and/or VLPs being able to elicit a cellular and/or humoral response against LASV, in particular a T cell response, in particular a CD4+ and/or CD8+ T cell response.

In particular, the invention is related to the use of these genetic constructs, recombinant nucleic acid constructs, expression vectors like plasmid vectors and the like, recombinant virus infectious particles, VLPs, for inducing an immunogenic or antigenic response within a host.

BACKGROUND OF THE INVENTION

Lassa Virus (LASV) is an old world arenavirus of the *Arenaviridae* family. LASV are enveloped, single-stranded, bisegmented ambisense RNA viruses. Their genome contains two RNA segments each coding for two proteins, one in each sense, for a total of four viral proteins. The larger segment (approximatively 7kb) encodes the zinc-binding protein Z. Z protein regulates replication and transcription. The larger segment also encodes the RNA polymerase L. The small segment (approximatively 3.4kb) encodes the nucleoprotein (NP) and the glycoprotein precursor (GPC), which is posttranslationnaly cleaved into the envelope glycoproteins GP1 and GP2 and the stable signal peptide SSP. These two glycoproteins mediate host cell entry. The synthesis capacity of an arenavirus is contained within the L polymerase protein. This protein uses viral RNA templates consisting in the genomic RNA encapsidated by the NP protein and viral ribonucleoproteins. Upon infection, the virus is delivered into the cytoplasm of the host cell, the L polymerase protein initiates transcription from the genome promoter located at the 3' end of each genomic RNA segment. The primary transcription results in the synthesis of mRNA of the viral genes encoded in the antigenomic orientation, *i.e.* of NP and L genes. Transcription terminates at the distal end of the stem-loop structure within the intergenomic region. Then, the L polymerase moves across the intergenomic region to generate a complementary antigenomic RNA. This RNA serves as a template for the synthesis of the mRNA of viral genes GPC and Z and for the synthesis of a full length genomic RNA.

LASV is the agent of the Lassa fever, a severe hemorrhagic fever, in humans. The natural reservoir of the virus is the African rodent *Mastomys natalensis*. Lassa virus is transmitted from rodents to humans, but the virus may also be transmitted from human to human, giving rise to local outbreaks.

Between 100.000 and 300.000 patients, and sometimes up to 500.000 patients, are reported with the Lassa fever each year in the endemic regions of west Africa, especially in Guinea, Liberia, Nigeria and Sierra Leone. Therefore, Lassa fever is a major public health concern in these regions. The severity of the disease varies from asymptomatic infection to severe complications leading to fatal hemorrhagic fever. Clinical signs and symptoms include fever, cough, chest pain, dysuria, headache, vomiting, diarrhea, pharyngitis, conjunctivitis, bleeding and facial edema. The mortality rates of patients infected with the LASV is high, with a rate around 10% in some areas where the LASV is distributed. The fatality rate is as high as 50% in young children. Moreover, approximatively 20% of the survivors present long-term complications including hearing deficit. Therefore, the Lassa fever has a serious impact on the population of these regions and is a major health problem. Lately, the distribution of the Lassa fever infections seems to spread in other west African countries, since cases have been reported in Mali, Ghana, Ivory Coast and Burkina Faso during the last decade.

Despite its discovery in 1969 in Nigeria, there is currently no preventive or prophylactic treatment against Lassa fever. Most of the patients are treated with Ribavirin, an antiviral drug. Unfortunately, treatment with the drug seems to be the most effective only when administrated early in the course of the illness and it is not fully efficient. The treatment should also be completed with supportive care, like maintenance of blood pressure and oxygenation, fluid and electrolyte balance, and the treatment of any other infections. Therefore, it may be difficult to effectively treat the patients in the endemic regions of west Africa where the Lassa fever spreads.

Whether infection leads to severe illness or death seems to depend on host immune response. Most of the severe cases include a defective cellular response, wherein the dendritic cells and macrophages massively release LASV but are not activated and therefore do not produce cytokines, or not enough. The disease severity, as much as the evolution and spread of the virus into new geographic areas, are a serious health public matter that needs to be fixed.

In this context, the development of a preventive treatment, like a preventive vaccine, is a major priority to meet the needs of these populations. There is therefore a need for a fully efficient treatment able to treat or prevent LASV infections, including to prevent outcomes of LASV primary infection, in particular to prevent the Lassa fever.

One of the most promising therapy for preventing LASV infections is prophylactic vaccination but no such vaccine is currently available. Prophylaxis would be the easiest and safest way to control the LASV infections, and protect the local populations. Despite this urgent need, no vaccine candidate has successfully advanced to clinical trials yet.

Therefore, there is a need for a vaccine and products such as active ingredients for preparing a vaccine, and method for producing these products and vaccine. The vaccine candidate should be safe and efficient when immunizing people in need thereof, without significant side effects, and induces the production of antibodies neutralizing the LASV, and possibly T cells like T helper cells and/or Cytotoxic T cells. In other words, the vaccine should elicit a strong cellular and/or humoral response. Advantageously, the vaccine should confer sterilizing immunity after a single immunization. To this end, there is a need for a vaccine that would enable the LASV proteins and/or LASV VLPs to generate *in vivo* in infected cells, in particular in infected cells of a host, and thus provide an efficient, long-lasting immunity, especially which induces life-long immunity after only a single, or two, administration steps.

Another need is to facilitate the vaccination of the populations that hardly have access to medical centers or the like. A vaccine candidate that would elicit immunization against two disease agents could enhance global health of these populations. A single vaccination could therefore allow the immunization against several disease agents present in the regions mentioned above. In particular, with the aim to totally eradicate the measles virus (MeV), a vaccine immunizing

against both the MeV and the LASV could clearly protect these populations against these two major threats, especially in west Africa.

Measles virus has been isolated in 1954 (Enders, J. F., and T. C. Peebles. 1954. Propagation in tissue cultures of cytopathogenic agents from patients with measles. Proc. Soc. Exp. Biol. Med.86:277-286.). Measles virus is a member of the order mononegavirales, *i.e.* viruses with a non-segmented negative-strand RNA genome. The non-segmented genome of MeV has an antimessage polarity which results in a genomic RNA which is neither translated *in vivo* or *in vitro* nor infectious when purified. Transcription and replication of non-segmented (-) strand RNA viruses and their assembly into virus particles have been studied and reported especially in Fields virology (3rd edition, vol. 1, 1996, Lippincott – Raven publishers – Fields BN *et al.*). Transcription and replication of the measles virus do not involve the nucleus of the infected cells but rather take place in the cytoplasm of host cell, just like the LASV. The genome of the MeV comprises genes encoding six major structural proteins designated N, P, M, F, H and L, and an additional two non-structural proteins from the P gene, C and V. The gene order is the following: from the 3' end of the genomic RNA; N, P (including C and V), M, F, H and L large polymerase at the 5' end. The genome furthermore comprises non coding regions in the intergenic region M/F. This non coding region contains approximatively 100 nucleotides of untranslated RNA. The cited genes respectively encode the proteins of the nucleocapsid of the virus or nucleoprotein (N), the phosphoprotein (P), the large protein (L) which together assemble around the genome RNA to provide the nucleocapsid, the hemagglutinin (H), the fusion protein (F) and the matrix protein (M).

Attenuated viruses have been derived from MeV virus to provide vaccine strains and in particular from the Schwarz strain. The Schwarz measles vaccine is a safe and efficient vaccine currently available for preventing measles. Besides providing vaccine, strains attenuated measles virus such as the Schwarz strain have shown to be stable and suitable for the design of efficient delivery vector for immunization against other viruses, like Zika virus or Chikungunya virus. Measles vaccines have been administered to hundreds of millions of children over the last

30 years and have proved its efficiency and safety. It is produced on a large scale in many countries and is distributed at low cost.

SUMMARY OF THE INVENTION

To address, at least partially, the drawbacks of the state of the art, the inventors achieved the production of active components (or ingredients) for vaccines based on recombinant genetic constructs, and especially based on recombinant nucleic acid constructs comprising, within an infectious replicative measles virus, cloned polynucleotide(s) encoding Lassa virus polypeptides, proteins or antigens, or antigenic fragments thereof. Vaccines may be recovered when the recombinant measles virus replicates in the host after administration. The invention thus relates to a LASV vaccine, especially a pediatric vaccine, and relates to active ingredient based on an attenuated measles virus strain such as a known vaccine strain commercially available, especially the widely used Schwarz measles vaccine. For all these reasons, the inventors used attenuated measles viruses to generate recombinant measles virus particles stably expressing structural antigens of LASV, in particular immunogenic particles thereof and/or VLPs. The measles approach of the invention meets all of the relevant criteria of a future LASV vaccine.

One aim of the invention is to provide a genetic construct, in particular recombinant genetic constructs, in particular nucleic acid constructs, for recovering infectious virus from the nucleic acid construct, and in particular a measles virus expressing LASV particles, and optionally also LASV Virus Like Particles (VLPs).

The invention therefor relates to a nucleic acid construct which comprises a cDNA molecule encoding the full-length antigenomic (+) RNA strand of the measles virus, and a first heterologous polynucleotide encoding at least one polypeptide, or at least one protein, or at least one antigen, or at least one antigenic fragment thereof, of the Lassa virus, said at least one polypeptide, or at least one protein, or at least one antigen, or at least one antigenic fragment thereof, being selected

from the group consisting of the nucleoprotein (NP), a mutated nucleoprotein (mNP), the Zinc-binding protein (Z) and the glycoprotein precursor (GPC). The first heterologous polynucleotide is operatively cloned within an additional transcription unit (ATU) inserted within the cDNA of the antigenomic (+) RNA strand of the MeV. Particular nucleic acid constructs according to this embodiment are illustrated in Fig.1 and in Fig. 31 to Fig. 36 and in the examples.

The expression “encodes” in the above definition encompasses the ability of the cDNA to allow transcription of a full length antigenomic (+) RNA, said cDNA serving especially as a template for transcription and where appropriate translation for product expression into cells or cell lines. Hence, when the cDNA is a double stranded molecule, one of the strands has the same nucleotide sequence as the antigenomic (+) RNA of the measles virus with the first heterologous polynucleotide cloned within, except “U” nucleotides that are substituted by “T” nucleotides in the cDNA. The nucleic acid construct of the invention may comprise regulatory elements controlling the transcription of the coding sequences, in particular promoters and termination sequences for the transcription, and possibly enhancer and other cis-acting elements. These regulatory elements may be heterologous with respect to the heterologous polynucleotide issued or derived from LASV gene(s), in particular may be the regulatory elements of the measles virus strain.

The expression “operatively cloned”, which can be substituted by the expression “operatively linked”, refers to the functional cloning, or insertion, of a heterologous polynucleotide within the nucleic acid construct of the invention such that said polynucleotide and nucleic acid construct are effectively, or efficiently, transcribed and if appropriate translated, in particular in cells, cell line, host cell used as a part of a rescue system for the production of recombinant infectious MeV particles or MeV expressing at least one polypeptide, or at least one protein, or at least one antigen, or at least an antigenic fragment thereof, of LASV. In other words, the nucleic acid construct of the invention allows the production, when placed in appropriate conditions, of an infectious antigenomic (+) RNA capable of

producing at least one polypeptide, or at least one protein, or at least one antigen, or at least an antigenic fragment thereof, of LASV.

In a particular embodiment of the invention, the nucleic acid construct comprising the cDNA encoding the nucleotides of the full-length infectious antigenomic (+) RNA strand of MeV but without the operatively cloned heterologous polynucleotide complies with the rule of six (6) of the measles virus genome. In other words, the cDNA encoding the nucleotides of the full-length, infectious antigenomic (+) RNA strand of MeV is a polyhexameric cDNA.

The organization of the genome of measles viruses and their replication and transcription process have been fully identified in the prior art and are especially disclosed in Horikami S.M. and Moyer S.A. (Curr. Top. Microbiol. Immunol. (1995) 191, 35-50 or in Combredet C. *et al* (Journal of Virology, Nov 2003, p11546-11554) for the Schwarz vaccination strain of the virus or for broadly considered negative-sense RNA viruses, in Neumann G. *et al* (Journal of General Virology (2002) 83, 2635-2662).

The “rule of six” is expressed in the fact that the total number of nucleotides present in a nucleic acid representing the MeV (+) strand RNA genome or in the nucleic acid constructs comprising the same is a multiple of six. The “rule of six” has been acknowledged in the state of the art as a requirement regarding the total number of nucleotides in the genome of the measles virus, which enables efficient or optimized replication of the MeV genomic RNA. In the embodiments of the present invention defining a nucleic acid construct that meets the rule of six, said rule applies to the nucleic acid construct specifying the cDNA encoding the full-length MV (+) strand RNA genome. In this regard the rule of six applies individually to the cDNA encoding the nucleotide sequence of the full-length infectious antigenomic (+) RNA strand of the measles virus, possibly but not necessarily to the polynucleotide cloned into said cDNA and encoding at least one polypeptide of the LASV.

The nucleic acid construct of the invention is in particular a purified DNA molecule, obtained or obtainable by recombination of at least one polynucleotide of MeV and at least one, or several, polynucleotide of the LASV, operably cloned or linked together.

According to the invention, the nucleic acid construct is prepared by cloning a polynucleotide, or several polynucleotides, encoding at least one polypeptide, or a protein, or an antigen, or an antigenic fragment thereof, selected from the group consisting of the GPC protein, the NP protein, the mNP protein and the Z protein of the LASV in the cDNA encoding the full-length antigenomic (+) RNA of the measles virus. The MeV genome is illustrated on Fig.1A, while several nucleic acid constructs according to the invention are illustrated on Fig.1B and on Fig. 31 to Fig. 36. Alternatively, a nucleic acid construct of the invention may be prepared using steps of synthesis of nucleic acid fragments or polymerization from a template, including by PCR. The polynucleotide(s) and nucleic acid construct of the invention may rather be prepared in accordance with any known method in the art and in particular may be cloned, obtained by polymerization especially using PCR methods or may be synthesized.

The heterologous polynucleotide may be issued from the fusion of several other polynucleotides, each encoding a particular polypeptide, or a particular protein, antigen or an antigenic fragment thereof, of LASV. For example, the heterologous polynucleotide may be issued from the fusion of polynucleotides each encoding a single protein, the GPC protein and the NP protein or the mNP protein for example, these two polynucleotides being linked within the nucleic acid construct by a linker sequence. A linker sequence is well known in the art and can be a short nucleotide sequence comprising or consisting in a regulatory sequence of the measles virus.

Accordingly, the heterologous polynucleotide may encode a single polypeptide, two different polypeptides, two identical polypeptides, three different polypeptides, two identical polypeptides and another polypeptide, four different polypeptides, two identical polypeptides and two others identical polypeptides,

two identical polypeptides and two others different polypeptides, three identical polypeptides and another different polypeptide. Any one of these polynucleotides encoding at least two (identical or different) polypeptides may be issued from the fusion of several polynucleotides, or prepared using steps of synthesis of nucleic acid fragments or polymerization from a template, including by PCR. Alternatively, any one of these polynucleotides may be a cDNA issued from the genomic RNA of the Lassa virus, after retrotranscription, said cDNA being either the full genomic cDNA or a fragment thereof, and encoding a polypeptide of the LASV.

The heterologous polynucleotide, in particular LASV gene(s), is/are cloned within an additional transcription unit (ATU) inserted in the cDNA of the MeV. ATU sequences are known from the skilled person and comprise, for use in steps of cloning into cDNA of MeV, cis-acting sequences necessary for MeV-dependent expression of a transgene, such as a promoter of the gene preceding, in MeV cDNA, the insert represented by the polynucleotide encoding the LASV polypeptide(s) inserted into a multiple cloning sites cassette. The ATU may be further defined as disclosed by Billeter *et al*. in WO 97/06270. Three ATUs are represented on Fig. 1A. An ATU may also be defined as multiple cloning cassette inserted within the cDNA of the MeV, in particular between the N-P intergenic region of the MeV genome, and/or between the intergenic H-L region of the MeV genome. An ATU may contain cis-acting sequences necessary for the transcription of the P gene of MeV. The different ATUs in particular ATU1 and ATU2 may be identical regarding their nucleic acid sequence. ATUs are generally localized between two CTT codons corresponding respectively to the start and stop codons of the polymerase. ATUs may further comprise a ATG and a TAG codons corresponding respectively to the start and stop codons for translation of the heterologous polynucleotide cloned within the ATU. Alternatively, ATUs are localized between a ATG and a TAG codons corresponding respectively to the start and stop codons for translation of the heterologous polynucleotide cloned within the ATU. ATUs may further comprise a ATG and a TAG codons corresponding respectively to the start and stop codons for translation of the heterologous polynucleotide cloned within the ATU. In a preferred embodiment

of the invention, an ATU is a polynucleotide comprising or consisting of SEQ ID No: 16.

SEQ ID No: 16

SEQ ID No: 16 is an ATU sequence localized within the cDNA molecule encoding a full-length antigenomic (+) RNA strand of a measles virus. CTT codons corresponding respectively to the start and stop codons of the polymerase are in bold. ATG and TAG codons corresponding to the start and stop codons for translation of the heterologous polynucleotide cloned within the ATU are underlined.

CTTAGGAACCAGGTCCACACAGCCGCCAGCCCATCAacgcgtacgATG*TAGg cgcgcagcgcttagacgtctcgcgaTCGATACTAGTACAACCTAAATCCATTATAAAAA A**CTT** wherein the * corresponds to the heterologous codon-optimized sequence polynucleotide encoding at least one LASV polypeptide.

An ATU with a heterologous polynucleotide encoding the GPC polypeptide is for example localized between amino acid residues 3487 and 5071 on SEQ ID No: 9. SEQ ID No: 17 corresponds to an ATU comprising as a cloned insert a codon-optimized heterologous polynucleotide encoding the GPC protein.

An ATU (known under reference ATU2) is localized between the P and M genes of the MeV. Another ATU (known under reference ATU1) is located upstream the gene N of the MeV. Another ATU (known under reference ATU3) is located between the genes H and L of MeV. It has been observed that the transcription of the viral RNA of MeV follows a gradient from the 5' to the 3' end. This explains that, depending on where the heterologous polynucleotide is inserted, its level of expression will vary and be more or less efficient if inserted within ATU1, ATU2 or ATU3.

The term "polypeptide" is used interchangeably with the terms "antigen" or "protein" or "antigenic fragment" and defines a molecule resulting from a concatenation of amino acid residues. In particular, the polypeptides disclosed in the application originate from the LASV and are antigens, proteins, structural

proteins, or antigenic fragments thereof, that may be identical to native proteins or alternatively that may be derived thereof by mutation, including by substitution (in particular by conservative amino acid residues) or by addition of amino acid residues or by secondary modification after translation or by deletion of portions of the native proteins(s) resulting in fragments having a shortened size with respect to the native protein of reference. Fragments are encompassed within the present invention to the extent that they bear epitopes of the native protein suitable for the elicitation of an immune response in a host in particular in a human host, including a child host, preferably a response that enables the protection against a LASV infection or against a LASV associated disease. Epitopes are in particular of the type of T epitopes involved in elicitation of Cell Mediated Immune response (CMI response). T epitopes are involved in the stimulation of T cells through presentation of some parts of the T-cell epitope which can bind on MHC class II molecules, leading to the activation of T cells. Epitopes may alternatively be of type B, involved in the activation of the production of antibodies in a host to whom the protein has been administered or in whom it is expressed following administration of the infectious replicative particles of the invention. Fragments may have a size representing more than 50% of the amino-acid sequence size of the native protein of LASV strain Josiah, preferably at least 90% or 95%. Polypeptide may have at least 50% identity with the native protein of LASV strain Josiah, preferably at least 60%, preferably at least 70%, preferably at least 85% or at least 95%.

In a particular embodiment of the invention, each polynucleotide operatively cloned within the cDNA of the antigenomic (+) RNA encodes polypeptides comprising epitopes localized within any one of the LASV polypeptide(s). According to this embodiment, the epitope sequence(s) share(s) 100% identity with the epitope sequence(s) of the native LASV proteins. Such epitopes are listed in the Immune Epitope database and analysis resource (www.iedb.org). Within the polypeptide(s) of the LASV encoded by the polynucleotide and having an epitope sequence(s) as defined herein, amino acid residue that does not belong to any epitope may be different from the sequence of the native LASV protein(s).

By “polypeptide of LASV” is meant a “polypeptide” as defined herein (either a polypeptide, an antigen, a protein, or an antigenic fragment thereof), the amino acid sequence of which is identical to a counterpart in a strain of LASV, especially LASV strain Josiah, including a polypeptide which is a native mature or precursor of protein of LASV or is an antigenic fragment thereof or a mutant thereof as defined herein in particular an antigenic fragment or a mutant having at least 50%, at least 80%, in particular advantageously at least 90% or preferably at least 95% amino acid sequence identity to a naturally occurring LASV GPC, NP or Z protein. Amino acid sequence identity can be determined by alignment by one of skill in the art using manual alignments or using the numerous alignment programs available (for example, BLASTP – http://blast.ncbi.nlm.nih.gov/). Fragments or mutants of LASV polypeptides of the invention may be defined with respect to the particular amino acid sequences illustrated herein, especially the amino acid sequences from the group consisting of SEQ ID No: 1, SEQ ID No: 3, SEQ ID No: 5 and SEQ ID No: 7. In a particular embodiment of the invention, the polypeptides share at least 50%, at least 80%, in particular advantageously at least 90% or preferably at least 95% amino acid sequence identity with their native proteins of the LASV strain Josiah, or with the polypeptides of SEQ ID No.: 1; SEQ ID No.: 3; SEQ ID No.: 5; or SEQ ID No.: 7.

According to one aspect of the invention, a polynucleotide encoding at least one polypeptide of LASV is issued or derived from the genome of isolated and purified wild strain(s) of LASV, including any Lassa strain whose genome has been fully or partially sequenced. At least some of these sequences may be found in the NCBI nucleotide database. In particular, the polynucleotide encoding at least one LASV polypeptide may be derived or issued from any Lassa strain sequenced and referenced in *Clinical Sequencing Uncovers Origins and Evolution of Lassa Virus* (Andersen Kristian G et al.; 2015; Cell. 2015 August 13; 162(4): 738–750. doi:10.1016/j.cell.2015.07.020), especially in the supplementary data of the publication wherein the name of the strains and corresponding accession reference of the sequences are listed. Preferentially, the polynucleotide is issued or derived from the strain Josiah whose genomic sequences of the two RNA

segments may be found under GenBank accession no. J04324.1 for the short genomic RNA encoding NP protein and GPC protein, and under European Nucleotide Archive accession no. U73034.2 for the long genomic RNA encoding the Z protein and the L protein. Native protein of LASV strain Josiah may be defined as having the sequences issued from RNA segments found under GenBank accession no. J04324.1 for the short genomic RNA encoding NP protein and GPC protein, and under European Nucleotide Archive accession no. U73034.2 for the long genomic RNA. The term “derive” appearing in the definition of the polynucleotides merely specifies that the sequence of said polynucleotide may be identical to the corresponding sequence in a LASV strain or may vary to the extent that it encodes polypeptides, antigens, proteins, or fragments thereof, of LASV that meet(s) the definition of the “polypeptide” according to the present invention. In particular, a polynucleotide derives from the nucleic acid of a LASV strain when it is codon-optimized with respect to such sequence. Accordingly, the term does not restrict the production mode of the polynucleotide.

Alternatively, fragments may be short polypeptides with at least 10 amino acid residues, which harbor epitope(s) of the native protein listed in the Immune Epitope database and analysis resource (www.iedb.org). Fragments in this respect also include polyepitopes.

According to another embodiment of the invention, the nucleic acid construct further comprises a second heterologous polynucleotide encoding at least one polypeptide, or an antigenic fragment thereof, of the LASV, said at least one polypeptide or antigenic fragment thereof being selected from the group consisting of the GPC protein, the NP protein, the mNP protein and Z protein, the second heterologous nucleotide being operatively cloned within another ATU at a location distinct from the location of the first cloned heterologous polynucleotide, preferentially upstream the N gene of the MeV, said another ATU being in particular the ATU1 inserted upstream the N gene of the MeV. The second heterologous polynucleotide or antigenic fragment thereof encodes in particular at least one polypeptide or antigenic fragment thereof different from the polypeptide(s) encoded by the first heterologous polynucleotide.

According to this embodiment, another ATU (known under reference ATU1) is advantageously located in the N-terminal sequence of the cDNA molecule encoding the full-length (+) RNA strand of the antigenome of the MeV upstream the N gene of MeV, while the other ATU (ATU2) is preferentially located between the P and M genes of the virus. Since the transcription of the viral RNA of MeV follows a gradient from the 5' to the 3' end, the inventors found that cloning two polynucleotides at different locations within the cDNA encoding the full-length antigenomic (+) RNA of the measles virus may lead to the production of higher yield of antigenic particles and/or LASV virus like particles (VLPs) when the Z protein is encoded by at least one heterologous polynucleotide, while this production may be less important when the polynucleotides are all cloned within a single and same location. Furthermore, cloning the heterologous polynucleotides at different locations may reduce the attenuation of the expression of the encoded polypeptides. Indeed, when several genes are cloned within a single ATU, it may lead to reduction of the expression of the encoded polypeptides. Particular nucleic acid constructs according to this embodiment are illustrated in Fig.1b and in the examples.

Within the other ATU, the second polynucleotide may encode any one of the previously listed polypeptide, or antigenic fragment thereof, of the LASV. Accordingly, the second polynucleotide localized within the other ATU may encode the same polypeptide(s) than the first polynucleotide inserted within the first ATU, or the second polynucleotide may encode at least one common polypeptide with the polypeptide(s) encoded by the first polynucleotide. In a preferred embodiment of the invention, the first polynucleotide and the second polynucleotide encodes at least one different polypeptide, or an antigenic fragment thereof.

The cDNA molecule encoding the full-length antigenomic (+) RNA strand of the MeV may be characteristic of or may be obtained from an attenuated strain of MeV. An "attenuated strain" of MeV is defined as a strain that is avirulent or less virulent than the parent strain in the same host, while maintaining immunogenicity

and possibly adjuvanticity when administered in a host for preserving immunodominant T and B cell epitopes and possibly the adjuvanticity such as the induction of T cell costimulatory proteins or cytokine IL-12.

An attenuated strain of a measles virus accordingly refers to a strain which has been serially passaged on selected cells and, possibly, adapted to other cells to produce seed strains suitable for the preparation of human vaccine strains, harboring a stable genome which would not allow reversion to pathogenicity nor integration in host chromosomes. As a particular “attenuated strain”, an approved strain for a vaccine is an attenuated strain suitable for the invention when it meets the criteria defined by the FDA (US Food and Drug Administration); *i.e.* it meets safety, efficacy, quality and reproducibility criteria, after rigorous reviews of laboratory and clinical data (www.fda.gov/cber/vaccine/vacappr.htm).

In particular, the cDNA molecule encoding the full-length antigenomic (+) RNA strand of the MeV is obtained from an attenuated virus strain selected from the group comprising of consisting of the Schwarz strain, the Zagreb strain, the AIK-C strain, the Moraten strain, the Philips strain, the Beckenham 4A strain, the Beckenham 16 strain, the Edmonston seed A strain, the Edmonston seed B strain, the CAM-70 strain, the TD 97 strain, the Leningrad-16 strain, the Shanghai 191 strain and the Belgrade strain. All these strains have been described in the prior art. The invention uses in particular strains that have been allowed for use as commercial vaccines. In particular, the cDNA molecule encoding the full length antigenomic (+) RNA strand of the MeV is obtained from the Schwarz strain.

According to a particular embodiment of the invention, the cDNA molecule is placed under the control of heterologous expression control sequences.

The insertion of such a control for the expression of the cDNA, is favorable when the expression of this cDNA is sought in cell types which do not enable full transcription of the cDNA with its native control sequences.

According to a particular embodiment of the invention, the heterologous expression control sequence comprises the T7 promoter and T7 terminator sequences. These sequences are respectively located 5' and 3' of the coding sequence for the full length antigenomic (+) RNA strand of MeV and from the adjacent sequences around this coding sequence.

In a particular embodiment of the invention, the cDNA molecule, which is defined here above is modified, *i.e.* comprises additional nucleotide sequences or motifs.

In a preferred embodiment, the cDNA molecule used according to the invention further comprises, at its 5'-end, adjacent to the first nucleotide of the nucleotide sequence encoding the full-length antigenomic (+) RNA strand of the MeV approved vaccine strain, a GGG motif followed by a hammerhead ribozyme sequence and comprises, at its 3'-end, adjacent to the last nucleotide of said nucleotide sequence encoding the full-length anti-genomic (+) RNA strand, the sequence of a ribozyme. The Hepatitis delta virus ribozyme (δ) is appropriate to carry out this preferred embodiment.

The GGG motif placed at the 5' end, adjacent to the first nucleotide of the above coding sequence improves the efficiency of the transcription of said cDNA coding sequence. As a requirement for the proper assembly of measles virus particles is the fact that the cDNA encoding the antigenomic (+) RNA complies with the rule of six, when the GGG motif is added, a ribozyme is also added at the 5' end of the coding sequence of the cDNA, 3' from the GGG motif, in order to enable cleavage of the transcript at the first coding nucleotide of the full-length antigenomic (+) RNA strand of MeV.

In order to prepare the nucleic acid construct of the invention, the preparation of a cDNA molecule encoding the full-length antigenomic (+) RNA of a measles virus disclosed in the prior art is achieved by known methods. The obtained cDNA provides especially the basis for the genome vector involved in the rescue of recombinant measles virus particles when it is inserted in a vector such as a plasmid.

A particular cDNA molecule suitable for the preparation of the nucleic acid construct of the invention is the one obtained using the Schwarz strain of measles virus. Plasmid pTM-MVSchw, which contains an infectious MeV cDNA corresponding to the anti-genome of the Schwarz MV vaccine strain and is used for preparation of recombinant vectors encompassing the heterologous polynucleotides of the invention, has been described elsewhere (Combredet, C., *et al*., A molecularly cloned Schwarz strain of measles virus vaccine induces strong immune responses in macaques and transgenic mice. J Virol, 2003. 77(21): p. 11546-54). Accordingly, the cDNA used within the present invention may be obtained as disclosed in WO2004/000876 or may be obtained from plasmid pTM-MVSchw deposited by Institut Pasteur at the CNCM under No I-2889 on June 12, 2002, the sequence of which is disclosed in WO2004/000876 incorporated herein by reference. The plasmid pTM-MVSchw has been obtained from a Bluescript plasmid and comprises the polynucleotide coding for the full-length measles virus (+) RNA strand of the Schwarz strain placed under the control of the promoter of the T7 RNA polymerase. It has 18967 nucleotides and a sequence represented as SEQ ID NO: 15. cDNA molecules (also designated cDNA of the measles virus or MeV cDNA for convenience) from other MeV strains may be similarly obtained starting from the nucleic acid purified from viral particles of attenuated MeV such as those described herein. An additional transcription unit may be a multiple-cloning site cassette previously inserted in the vector, as explained in Combredet *et al*., 2003. An ATU may comprise *cis*-acting sequences necessary for the transcription of the inserted LASV genes. The heterologous polynucleotide(s) are cloned or inserted within additional transcription units (ATU) as defined here above.

The heterologous polynucleotide may also be cloned or inserted within another ATU. As an example, a third ATU may be inserted between the H gene and the L gene of the MeV, and the first or second heterologous polynucleotide may be cloned or inserted within this third ATU. In a particular embodiment of the invention, the nucleic acid construct may comprise a first heterologous polynucleotide inserted within a first ATU, a second heterologous polynucleotide

sequence inserted into a second ATU at a distinct location from the first ATU, and a third heterologous polynucleotide inserted within a third ATU at a distinct location from the first and second ATUs.

In a preferred embodiment, the nucleic acid construct comprises heterologous polynucleotide(s) encoding a particular mutated NP protein, or an antigenic fragment thereof. The native NP protein may be mutated to knock down the exonuclease activity of the NP protein. A NP protein knocked down for its exonuclease activity may be determined by luciferase assay using a reporter luciferase gene placed under the control of an IRF3 dependent promoter as described in PMID: 21085117 and illustrated on Fig. 29. NP is involved in the virus-induced inhibition of type I IFN signaling (Martinez-Sobrido, 2006). This activity is linked to the C-terminal domain of the native NP protein. Functional analysis confirmed the exonuclease activity of LASV NP, which has been proven to be a critical step for its type I IFN counteracting function (Qi, 2010). Hence, the inventors introduced two mutations within the exonuclease domain of the NP protein. The exonuclease domain of the NP protein is localized within the C-terminal domain of the NP protein, especially between amino acid residues 341 and 569 of SEQ ID no: 3. Accordingly, a mutated NP protein is in particular issued from a native NP protein mutated (by deletion(s) and/or addition(s) and/or substitution(s) of any amino acid residue) within the exonuclease domain as defined here above, and having its exonuclease activity knocked down according to the luciferase assay described here above. In particular, substitution(s) of at least one amino acid residue D389, E391, D466, D533 and H528 of SEQ ID No: 3 may lead to a mutated NP protein without exonuclease activity. In particular, the amino acid residue at position 389 of SEQ ID No: 3 may be mutated, for example by substituting the Aspartic acid by an Alanine. Alternatively, amino acid residue at position 392 of SEQ ID No: 3 may be mutated, for example by substitution of the Glycine by an Alanine. In a preferred embodiment, both amino acids residues at position 389 and 392 of SEQ ID No.3 are mutated by substitution. In a more preferred embodiment, the mNP protein has the sequence of SEQ ID No: 5.

According to this embodiment, the mNP polypeptide encoded by the polynucleotide(s) allows the induction of type I IFN. In other words, the immune suppressive function of the native NP is shut down in mNP. Therefore, it is provided nucleic acid constructs comprising polynucleotide(s) which increase the efficiency of chimeric recombinant MeV-LASV infectious particles immunogenicity.

According to a preferred embodiment, the invention also concerns modification and in particular optimization of the polynucleotides to allow an efficient expression of the LASV polypeptides, proteins, antigens, or fragments thereof, in a host cell.

Accordingly, optimization of the polynucleotide sequence can be operated avoiding cis-active domains of nucleic acid molecules: internal TATA-boxes, chi-sites and ribosomal entry sites; AT-rich or GC-rich sequence stretches; ARE, INS, CRS sequence elements; repeat sequences and RNA secondary structures; cryptic splice donor and acceptor sites, branch points.

The optimized polynucleotides may also be codon optimized for expression in a specific cell type, in particular may be modified for the Maccaca codon usage or for the human codon usage. This optimization allows increasing the efficiency of chimeric infectious particles production in cells without impacting the amino acid composition of the expressed protein(s).

In particular, the optimization of the polynucleotide encoding the LASV polypeptide(s) may be performed by modification of the wobble position in codons without impacting the identity of the amino acid residue translated from said codon with respect to the original one.

Optimization is also performed to avoid editing-like sequences from Measles virus. The editing of transcript of measles virus is a process which occurs in particular in the transcript encoded by the P gene of measles virus. This editing, by the insertion of extra G residues at a specific site within the P transcript, gives

rise to a new protein truncated compared to the P protein. Addition of only a single G residue results in the expression of the V protein, which contains a unique carboxyl terminus (Cattaneo R *et al*., Cell. 1989 Mar 10;56(5):759-64).

In the polynucleotides according to this particular embodiment of the invention, the following editing-like sequences from measles virus can be mutated: AAAGGG, AAAAGG, GGGAAA, GGGGAA, as well as their complementary sequence: TTTCCC, TTTTCC, CCCTTT, CCCCTT. For example, AAAGGG can be mutated in AAAGGC, AAAAGG can be mutated in AGAAGG or in TAAAGG or in GAAAGG, and GGGAAA in GCGAAA.

Hence, the heterologous polynucleotide(s) may comprise any one of the following sequences, or a plurality of the following sequences, or at least two of the following sequences, or at least three of the following sequences, or the four following sequences:

- SEQ ID No: 2 which encodes the GPC protein; and/or
- SEQ ID No: 4 which encodes the NP protein; and/or
- SEQ ID No: 6 which encodes the mNP protein; and/or
- SEQ ID No: 8 which encodes the Z protein.

Within the heterologous polynucleotide(s), each sequence defined here above may be present a single time, or a plurality of times. In a preferred embodiment of the invention, each sequence is present a single time within a single heterologous polynucleotide, or is present a single time within the heterologous polynucleotides taken together.

According to any one of the particular embodiments of the invention, it is provided nucleic acid constructs comprising polynucleotide(s) which increase the efficiency of chimeric recombinant MeV-LASV infectious particles production.

Alternatively, or complementarily, the heterologous polynucleotide(s) may encode any one of the following polypeptides, or an antigenic fragment thereof, or at least two of the following polypeptides, or at least three of the following polypeptides, or the four following polypeptides:

- the GPC protein of SEQ ID No: 1 or an antigenic fragment thereof; and/or
- the NP protein of SEQ ID No: 3 or an antigenic fragment thereof; and/or
- the mNP protein of SEQ ID No: 5 or an antigenic fragment thereof; and/or
- the Z protein of SEQ ID No: 7 or an antigenic fragment thereof.

It should be noted that the polynucleotide(s) may encode a polypeptide as defined here above a single time or a several times. In a preferred embodiment, each polypeptide is encoded a single time within a single heterologous polynucleotide, and more preferentially, each polypeptide is encoded a single time within the plurality of polypeptides. According to a particular embodiment of the invention, several polynucleotides wherein each polynucleotide encodes at least one LASV protein are combined or fused to form a polynucleotide encoding several proteins of the LASV. These polynucleotides may distinguish from each other by the fact that they code for proteins of various strains of the LASV, or for different proteins of a LASV strain.

In a particular embodiment, the nucleic acid construct of the invention comprises from the 5' to 3' end the following polynucleotides:

(a) a polynucleotide encoding the N protein of the MeV;

(b) a polynucleotide encoding the P protein of the MeV;

(c) the first heterologous polynucleotide encoding at least one polypeptide selected from the group consisting of the GPC protein, the NP protein, the mNP protein and the Z protein of the LASV, or an antigenic fragment thereof, in particular encoding a single polypeptide which is the GPC protein or an antigenic fragment thereof, or encoding at least two polypeptides, which are the GPC protein or an antigenic fragment thereof and either the NP protein or the mNP protein, or an antigenic fragment thereof, in particular encoding the GPC protein and the mNP protein, wherein the first polynucleotide is in particular operatively cloned within an ATU, in particular ATU2;

(d) a polynucleotide encoding the M protein of the MeV;

(e) a polynucleotide encoding the F protein of the MeV;

(f) a polynucleotide encoding the H protein of the MeV;

(g) a polynucleotide encoding the L protein of the MeV;

and wherein said polynucleotides are operatively linked within the nucleic acid construct and under the control of a viral replication and transcriptional regulatory elements such as MeV leader and trailer sequence(s).

Several examples of this embodiment obtained according to the invention are schematically illustrated on fig. 1B: the constructs named MeV-GPC_{LASV}; MeV-NP+GPC_{LASV}; MeV-NP_{ExoN}+GPC_{LASV} belong to this particular embodiment, but other construct not illustrated on Fig.1B are also included, like for example MeV-GPC_{LASV}+NP; MeV-GPC_{LASV}+NP_{ExoN}; MeV-Z+GPC_{LASV}; MeV-GPC_{LASV}+Z; MeV-Z+NP; MeV-Z+NP_{ExoN}; MeV-NP+Z; MeV-NP_{ExoN}+Z; MeV-Z+GPC_{LASV}+NP; MeV-Z+GPC_{LASV}+NP_{ExoN}; MeV-Z-NP-GPC_{LASV}; MeV-Z-NP_{ExoN}-GPC_{LASV}; MeV-GPC_{LASV}+NP+Z; MeV-GPC_{LASV}+NP_{ExoN}+Z; MeV-GPC_{LASV}+Z+NP; MeV-GPC_{LASV}+Z+NP_{ExoN}; MeV-NP+GPC_{LASV}+Z; MeV-NP-Z+GPC_{LASV}; MeV-NP_{ExoN}+GPC_{LASV}+Z; MeV-NP_{ExoN}-Z+GPC_{LASV};
wherein
MeV corresponds to the cDNA molecule encoding the full-length antigenomic (+) RNA strand of the measles virus (MeV);
GPC_{LASV} corresponds to a polynucleotide encoding a polypeptide of the GPC protein, or an antigenic fragment thereof;
NP corresponds to a polynucleotide encoding a polypeptide of the NP protein, or an antigenic fragment thereof;
NP_{ExoN} corresponds to a polynucleotide encoding a polypeptide of the mNP protein, or an antigenic fragment thereof;
Z corresponds to a polynucleotide encoding a polypeptide of the Z protein, or an antigenic fragment thereof;

The expressions "N protein", "P protein", "M protein", "F protein", "H protein" and "L protein" refer respectively to the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the hemagglutinin protein (H) and the RNA polymerase large protein (L) of a measles virus and encompass reference

to the respective polypeptides or antigenic fragments thereof. These components have been identified in the prior art and are especially disclosed in Fields, Virology (Knipe & Howley, 2001).

In another particular embodiment of the invention, the nucleic acid construct comprises from the 5' to 3' end the following polynucleotides:

(a) the second heterologous polynucleotide encoding at least one polypeptide selected from the group consisting of the GPC protein, the NP protein, the mNP protein and the Z protein of the LASV, or an antigenic fragment thereof, in particular encoding the Z protein, or an antigenic fragment thereof, wherein the second polynucleotide is operatively cloned within an ATU localized upstream the N gene of the MeV, in particular within the ATU1;

(b) a polynucleotide encoding the N protein of the MeV;

(c) a polynucleotide encoding the P protein of the MeV;

(d) the first heterologous polynucleotide encoding at least one polypeptide selected from the group consisting of the GPC protein, the NP protein, the mNP protein and the Z protein of the LASV, or an antigenic fragment thereof, in particular encoding a single polypeptide which is the GPC protein or an antigenic fragment thereof, or encoding at least two polypeptides, which are the GPC protein or an antigenic fragment thereof and either the NP protein or the mNP protein, or an antigenic fragment thereof, in particular encoding the GPC protein and the mNP protein, wherein the first polynucleotide is in particular operatively cloned within an ATU, in particular ATU2;

(e) a polynucleotide encoding the M protein of the MeV;

(f) a polynucleotide encoding the F protein of the MeV;

(g) a polynucleotide encoding the H protein of the MeV;

(h) a polynucleotide encoding the L protein of the MeV,

and wherein said polynucleotides are operatively linked within the nucleic acid construct and under the control of a viral replication and transcriptional regulatory elements such as MeV leader and trailer sequence(s).

Several examples of this embodiment are schematically illustrated on fig. 1B: the constructs named Z-MeV-GPC_{LASV}; Z-MeV-NP+GPC_{LASV}; Z-MeV-NP_{ExoN}+GPC_{LASV} are encompassed within this particular embodiment. When the protein of LASV is named before MeV, said protein is cloned within the additional transcription unit localized upstream the N gene of the MeV. It should be noted that several non-represented constructs are also encompassed by this embodiment. As an example, a heterologous polynucleotide may be cloned within the third ATU. Nucleic acid constructs corresponding to Z-MeV-GPC_{LASV}(ATU2)+NP(ATU3), or Z-MeV- NP(ATU2)- GPC_{LASV}(ATU3), or Z-MeV-GPC_{LASV}(ATU2)+mNP(ATU3), or Z-MeV- mNP(ATU2)- GPC_{LASV}(ATU3) are also encompassed by the present invention. It should be noted that a heterologous polynucleotide encoding at least one or any one of the Z polypeptide, the GPC polypeptide and/or the NP or mNP polypeptide could be inserted within ATU3. The various terms used therein have the same meaning as the one used in the previous particular embodiments.

In a particular embodiment of the invention, the nucleic acid construct comprises within the first heterologous polynucleotide a nucleic acid encoding the NP protein, preferentially the NP protein of SEQ ID No: 3, or an antigenic fragment thereof; and a nucleic acid encoding the GPC protein, preferentially the GPC protein of SEQ ID No: 1. In a preferred embodiment, this first heterologous polynucleotide is cloned between the P and M genes of the MeV, preferentially within ATU2 as defined here above.

In a particular embodiment of the invention, the nucleic acid construct comprises within the first heterologous polynucleotide a nucleic acid of SEQ ID No: 4 encoding the NP protein, and a nucleic acid of SEQ ID No: 2 encoding the GPC protein, preferentially these two nucleic acids are separated by a linker sequence. In a preferred embodiment, the nucleic acid of SEQ ID No: 4 is localized upstream the nucleic acid of SEQ ID No: 2. This is for example illustrated on Fig.1B with the construct named MeV-NP+GPC_{LASV}.

In a particular embodiment of the invention, the nucleic acid construct comprises within the first heterologous polynucleotide a nucleic acid encoding the mNP protein, preferentially the mNP protein of SEQ ID No: 5, or an antigenic fragment thereof; and a nucleic acid encoding the GPC protein, preferentially the GPC protein of SEQ ID No: 1.

In a particular embodiment of the invention, the nucleic acid construct comprises within the first heterologous polynucleotide a nucleic acid of SEQ ID No: 6 encoding the mNP protein, and a nucleic acid of SEQ ID No: 2 encoding the GPC protein, preferentially these two nucleic acids are separated by a linker sequence. In a preferred embodiment, the nucleic acid of SEQ ID No: 6 is located upstream (towards the 5' end of the construct) the nucleic acid of SEQ ID No: 2, as illustrated in Fig.1B with the construct named MeV-NP_{ExoN}+GPC_{LASV}. In a preferred embodiment, this first heterologous polynucleotide is cloned between the P and M genes of the MeV, preferentially within ATU2 as defined here above.

In a particular embodiment of the invention, the nucleic acid construct comprises the first heterologous polynucleotide and the second heterologous polynucleotide, and:

- the second heterologous polynucleotide comprises a nucleic acid encoding the Z protein, or an antigenic fragment thereof, preferentially the Z protein of SEQ ID No: 7; the second heterologous polynucleotide being preferentially cloned within ATU1 as defined here above, and
- the first heterologous polynucleotide comprises a nucleic acid encoding the GPC protein, or an antigenic fragment thereof, preferentially the GPC protein of SEQ ID No: 1, the first heterologous polynucleotide being preferentially cloned within ATU2 as defined here above.

In a particular embodiment of the invention, the nucleic acid construct comprises the first heterologous polynucleotide and the second heterologous polynucleotide, and:

- the second heterologous polynucleotide comprises a nucleic acid of SEQ ID No: 8 encoding the Z protein, the second heterologous

polynucleotide being preferentially cloned within ATU1 as defined here above, and

- the first heterologous polynucleotide comprises a nucleic acid of SEQ ID No: 2 encoding the GPC protein, the first heterologous polynucleotide being preferentially cloned within ATU2 as defined here above.

In a particular embodiment of the invention, the nucleic acid construct comprises a recombinant cDNA whose sequence is selected from the group consisting of:

- SEQ ID No: 9 (MeV-GPC);
- SEQ ID No: 10 (MeV-NP-GPC);
- SEQ ID No: 11 (MeV-mNP-GPC);
- SED ID No: 12 (Z-MeV-GPC);
- SEQ ID No: 13 (Z-MeV-NP-GPC); and
- SEQ ID No: 14 (Z-MeV-mNP-GPC),

wherein said sequences are described as follows:

SEQ ID NO: 9

SEQ ID No: 9 is the sequence of a nucleic acid construct according to a particular embodiment of the invention wherein said construct contains the pTM1-MVSchwarz vector wherein the sequence encoding the GPC protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2.

SEQ ID NO: 10

SEQ ID No: 10 is the sequence of a nucleic acid construct according to another particular embodiment of the invention wherein said construct contains the pTM1-MVSchwarz vector wherein the sequence encoding the GPC protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2, and wherein the sequence encoding the NP protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2.

SEQ ID NO: 11

SEQ ID No: 11 is the sequence of a nucleic acid construct according to another particular embodiment of the invention wherein said construct contains the pTM1-MVSchwarz vector wherein the sequence encoding the GPC protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2, and

wherein the sequence encoding the mutated NP protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2.

SEQ ID NO: 12

SEQ ID No: 12 is the sequence of a nucleic acid construct according to another particular embodiment of the invention wherein said construct contains the pTM1-MVSchwarz vector wherein the sequence encoding the GPC protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2, and wherein the sequence encoding the Z protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 1.

SEQ ID NO: 13

SEQ ID No: 13 is the sequence of a nucleic acid construct according to another particular embodiment of the invention wherein said construct contains the pTM1-MVSchwarz vector wherein the sequence encoding the GPC protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2, and wherein the sequence encoding the NP protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2, and wherein the sequence encoding the Z protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 1.

SEQ ID NO: 14

SEQ ID No: 14 is the sequence of a nucleic acid construct according to another particular embodiment of the invention wherein said construct contains the pTM1-MVSchwarz vector wherein the sequence encoding the GPC protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2, and wherein the sequence encoding the mutated NP protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 2, and wherein the sequence encoding the Z protein of LASV strain Josiah has been cloned within the Additional Transcription Unit 1.

The invention also relates to a transfer vector, which may be used for the preparation of recombinant MeV-LASV particles when rescued from helper cells or production cells. Several transfer vectors are illustrated on Fig. 31 to 36. In a preferred embodiment of the invention, the transfer vector is a transfer vector plasmid suitable for the transfection of helper cells or of production cells, and

comprising the nucleic acid construct according to the invention. The transfer vector plasmid may be obtained from a Bluescript plasmid and may be obtained by cloning the heterologous polynucleotide(s) of the invention in the pTM-MVSchw plasmid described here above. In particular embodiments of the invention, the transfer plasmid vector has the sequence of SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, SEQ ID No: 12, SEQ ID No: 13 or SEQ ID No: 14.

The invention also concerns the use of a transfer plasmid vector or the use of the nucleic acid construct according to the invention to transform cells suitable for the rescue of recombinant viral MeV-LASV particles, in particular to transfect or to transduce such cells respectively with plasmids or with viral vectors harboring the nucleic acid construct of the invention, said cells being selected for their capacity to express required measles virus proteins for appropriate replication, transcription and encapsidation of the recombinant genome of the virus corresponding to the nucleic acid construct of the invention in recombinant, infectious, replicative recombinant MeV-LASV particles.

The nucleic acid construct of the invention and the transfer plasmid vector are suitable and intended for the preparation of recombinant infectious replicative recombinant measles - Lassa virus (MeV-LASV) and accordingly said nucleic acid construct and transfer plasmid vector are intended for insertion in a transfer genome vector that as a result comprises the cDNA molecule of the measles virus, especially of the Schwarz strain, for the production of said recombinant MeV-LASV virus and expression of LASV polypeptide(s), possibly as LASV VLPs when the Z protein is encoded by at least one heterologous polynucleotide. The pTM-MVSchw plasmid is suitable to prepare the transfer vector, by insertion of the heterologous polynucleotide(s) as described herein necessary for the expression of LASV polypeptide(s), protein(s), antigen(s), or antigenic fragment(s) thereof. As used herein, the term "virus-like particle" (VLP) refers to a structure that in at least one attribute resembles a virus but which has not been demonstrated to be infectious as such. Virus Like Particles in accordance with the invention do not carry genetic information encoding the proteins of the Virus Like Particles, in general, virus-like particles lack a viral genome and, therefore,

are noninfectious and non- replicative. In accordance with the present invention, Virus Like Particles can be produced in large quantities and are expressed together with MeV-LASV recombinant particles.

The invention also relates to the cells or cell lines thus transformed by the transfer vector of the invention and by further polynucleotides providing helper functions and proteins. Polynucleotides are thus present in said cells, which encode proteins that include in particular the N, P and L proteins of a measles virus (*i.e.*, native MeV proteins or functional variants thereof capable of forming ribonucleoprotein (RNP) complexes), preferably as stably expressed proteins at least for the N and P proteins functional in the transcription and replication of the recombinant viral MeV-LASV particles. The N and P proteins may be expressed in the cells from a plasmid comprising their coding sequences or may be expressed from a DNA molecule inserted in the genome of the cell. The L protein may be expressed from a different plasmid. It may be expressed transitory. The helper cell is also capable of expressing a RNA polymerase suitable to enable the synthesis of the recombinant RNA derived from the nucleic acid construct of the invention, possibly as a stably expressed RNA polymerase. The RNA polymerase may be the T7 phage polymerase or its nuclear form (nlsT7).

In an embodiment, the cDNA clone of a measles virus is from the same measles virus strain as the N protein and/or the P protein and/or the L protein. In another embodiment, the cDNA clone of a measles virus is from a different strain of virus than the N protein and/or the P protein and/or the L protein.

The cells transformed or transfected with a nucleic acid construct according to the invention are able to produce recombinant measles viruses and and/or LASV VLPs when the Z protein is encoded by at least one heterologous polynucleotide. Accordingly, the recombinant measles virus comprises in its genome the nucleic acid construct of the invention and is able to express at least one polypeptide, protein or antigenic fragment thereof, of the LASV. Hence, the measles virus of the invention is able to express the GPC protein, or the GPC polypeptide, or an antigenic fragment thereof; and/or the NP protein, or the NP polypeptide, or an

antigenic fragment thereof; and/or the mNP protein, or the mNP polypeptide, or an antigenic fragment thereof; and/or the Z protein, or the Z polypeptide, or an antigenic fragment thereof. LASV VLPs comprise at least the Z protein, or an antigenic fragment thereof, and may further comprise one other polypeptide of LASV; the GPC protein, a fragment of the GPC protein like GP1 or GP2, the NP protein and/or the mNP protein. In a preferred embodiment, the LASV VLPs comprise the Z protein, or an antigenic fragment thereof, and the GPC protein, or an antigenic fragment thereof, or a fragment of the GPC protein like GP1 and/or GP2. In another preferred embodiment, the LASV VLPs comprise the Z protein, or an antigenic fragment thereof, the GPC protein, or an antigenic fragment thereof, and the mNP protein or the NP protein, or an antigenic fragment thereof.

In a preferred embodiment of the invention, the recombinant measles virus expresses the GPC protein and the mNP protein of the LASV. In another preferred embodiment of the invention, the recombinant measles virus expresses the GPC protein and the Z protein of LASV.

Furthermore, according to some embodiments of the invention, the recombinant measles virus also expresses at least one polypeptide or protein, or an antigenic fragment thereof, of the measles virus. In other words, the recombinant measles virus expresses at least one of the following polypeptides: the N protein, the P protein, the M protein, the F protein, the H protein and the L protein of the MeV.

According to this embodiment, the recombinant virus expresses recombinant antigenic particles of the measles virus and the Lassa virus, allowing the elicitation of cellular response, or a humoral response, or a cellular and humoral response against polypeptides of the LASV and against polypeptides of the MeV. In particular embodiments of the invention, the elicitation of the cellular response comprises elicitation of a T cell response, in particular CD4+ and/or CD8+ T cells response.

The invention thus relates to a process for the preparation of recombinant infectious measles virus particles comprising:

(a) transfecting cells, in particular helper cells, in particular HEK293 helper cells, stably expressing T7 RNA polymerase and measles N and P proteins with the nucleic acid construct according to the invention or with the transfer plasmid vector according to the invention;

(b) maintaining the transfected cells in conditions suitable for the production of recombinant measles virus and/or LASV VLPs;

(c) infecting cells enabling propagation of the recombinant measles virus and/or the LASV VLPs by co-cultivating them with the transfected cells of step (b);

(d) harvesting the recombinant measles virus expressing at least one LASV protein, preferentially at least the GPC protein and optionally the NP protein, the mNP protein and/or the Z protein, preferentially expressing the GPC protein and the mNP protein, and/or the LASV VLPs expressing at least the Z protein and possibly another LASV protein selected from the group consisting of the GPC protein, the NP protein and/or the mNP protein.

According to a particular embodiment, the invention relates to a process for the preparation of recombinant infectious measles virus particles comprising:

a) transferring, in particular transfecting, the nucleic acid construct of the invention or the transfer vector containing such nucleic acid construct in a helper cell line which also expresses proteins necessary for transcription, replication and encapsidation of the antigenomic (+) RNA sequence of MeV from its cDNA and under conditions enabling viral particles assembly and

b) recovering the recombinant infectious MeV-LASV virus expressing at least one polypeptide or protein of LASV, or an antigenic fragment thereof.

According to a particular embodiment of the invention, the process comprises:

a) transfecting helper cells with a nucleic acid construct according to the invention with a transfer plasmid vector, wherein said helper cells are capable of expressing helper functions to express an RNA polymerase, and to express the N, P and L proteins of a MeV virus;

b) co-cultivating said transfected helper cells of step 1) with passaged cells suitable for the passage of the MeV attenuated strain from which the cDNA originates;

c) recovering the recombinant infectious MeV-LASV virus expressing at least one polypeptide of the LASV.

According to another particular embodiment of the invention the method for the production of recombinant infectious MeV-LASV comprises:

a) recombining a cell or a culture of cells stably producing a RNA polymerase, the nucleoprotein (N) of a measles virus and the polymerase cofactor phosphoprotein (P) of a measles virus, with a nucleic acid construct of the invention and with a vector comprising a nucleic acid encoding a RNA polymerase large protein (L) of a measles virus, and

b) recovering the infectious, MeV-LASV virus from said recombinant cell or culture of recombinant cells.

According to a particular embodiment of the process, recombinant MeV are produced, which express LASV protein(s) comprising at least the GPC protein and/or LASV VLPs comprising at least the Z protein, and wherein the recombinant MeV and/or VLPs may express at least one other LASV proteins, or antigen, or an antigenic fragment thereof, e.g. GPC or a fragment thereof, especially GP1 and/or GP2, and optionally mNP of LASV. In other embodiment, the LASV VLPs comprise the Z protein or a fragment thereof, and optionally the GPC protein, and possibly GP1 and/or GP2. In a preferred embodiment of the invention, the LASV VLPs comprise the Z protein, or antigenic fragment thereof, and the GPC protein, or an antigenic fragment thereof. As an illustration, a process to rescue recombinant MeV expressing LASV proteins, in particular LASV VLPs comprises the steps of:

1) cotransfecting helper cells, in particular HEK293 helper cells, that stably express T7 RNA polymerase, and measles N and P proteins with (i) a transfer vector, in particular a plasmid, comprising cDNA encoding the full-length antigenomic (+) RNA of a measles virus recombined with at least one

polynucleotide encoding at least one LASV protein, for example encoding the GPC protein, the NP protein, the mNP protein and/or the Z protein, and with (ii) a vector, especially a plasmid, encoding the MeV L polymerase cDNA;

2) cultivating said cotransfected helper cells in conditions enabling the production of MV-LASV recombinant virus;

3) propagating the thus produced recombinant virus by co-cultivating said helper cells of step 2) with cells enabling said propagation such as Vero cells;

4) recovering replicating MeV-LASV recombinant virus and LASV protein(s), in particular LASV Virus Like Particles.

As used herein, “recombining” means introducing at least one polynucleotide into a cell, for example under the form of a vector, said polynucleotide integrating (entirely or partially) or not integrating into the cell. According to a particular embodiment, recombination can be obtained with a first polynucleotide, which is the nucleic acid construct of the invention. Recombination can, also or alternatively, encompasses introducing a polynucleotide, which is a vector encoding a RNA polymerase large protein (L) of a measles virus, whose definition, nature and stability of expression has been described herein.

In accordance with the invention, the cell or cell lines or a culture of cells stably producing a RNA polymerase, a nucleoprotein (N) of a measles virus and a polymerase cofactor phosphoprotein (P) of a measles virus is a cell or cell line as defined in the present specification or a culture of cells as defined in the present specification, *i.e.*, are also recombinant cells to the extent that they have been transformed by the introduction of one or more polynucleotides as defined above. In a particular embodiment of the invention, the cell or cell line or culture of cells, stably producing the RNA polymerase, the N and P proteins, does not produce the L protein of a measles virus or does not stably produce the L protein of a measles virus, *e.g.*, enabling its transitory expression or production. The production of recombinant MeV-LASV virus of the invention may involve a transfer of cells transformed as described herein. “Transfer” as used herein refers to the plating of the recombinant cells onto a different type of cells, and particularly onto monolayers of a different type of cells. These latter cells are competent to

sustain both the replication and the production of infectious recombinant MeV-LASV virus *i.e.*, respectively the formation of infectious viruses inside the cell and possibly the release of these infectious viruses outside of the cells possibly with release of LASV immunogenic particles and/or LASV VLPs. This transfer results in the co-culture of the recombinant cells of the invention with competent cells as defined in the previous sentence. The above transfer may be an additional, *i.e.*, optional, step when the recombinant cells are not efficient virus-producing culture *i.e.*, when infectious recombinant MeV-LASV virus cannot be efficiently recovered from these recombinant cells. This step is introduced after further recombination of the recombinant cells of the invention with any nucleic acid construct of the invention, and optionally a vector comprising a nucleic acid encoding a RNA polymerase large protein (L) of a measles virus.

In a particular embodiment of the invention, a transfer step is required since the recombinant cells, usually chosen for their capacity to be easily recombined are not efficient enough in the sustaining and production of recombinant infectious MeV-LASV virus. In said embodiment, the cell or cell line or culture of cells of step 1) of the above-defined methods is a recombinant cell or cell line or culture of recombinant cells according to the invention.

Cells suitable for the preparation of the recombinant cells of the invention are prokaryotic or eukaryotic cells, particularly animal or plant cells, and more particularly mammalian cells such as human cells or non-human mammalian cells or avian cells or yeast cells. In a particular embodiment, cells, before recombination of its genome, are isolated from either a primary culture or a cell line. Cells of the invention may be dividing or non-dividing cells.

According to a preferred embodiment, helper cells are derived from human embryonic kidney cell line 293, which cell line 293 is deposited with the ATCC under No. CRL-1573. Particular cell line 293 is the cell line disclosed in WO2008/078198 and referred to in the following examples as 293T7/N/P. Thus, the invention also relates to a host cell, in particular an avian cell or a mammalian cell, transfected or transformed with the nucleic acid construct according to any

embodiment of the invention, or transfected with a transfer plasmid vector. Suitable cells are the VERO NK cells (African green monkey kidney cells), and MRC5 cells (Medical Research Council cell strain 5). According to another aspect of this process, the cells suitable for passage are CEF cells (chick embryo fibroblasts). CEF cells can be prepared from fertilized chicken eggs as obtained from EARL Morizeau (8 rue Moulin, 28190 Dangers, France) or from any other producer of fertilized chicken eggs.

The process which is disclosed according to the present invention is used advantageously for the production of infectious replicative recombinant MeV-LASV virus appropriate for use as immunization compositions. The invention thus relates to a composition, in particular an antigenic composition, whose active principle comprises infection replicative recombinant MeV-LASV virus rescued from the nucleic acid construct of the invention and in particular obtained by the process disclosed. The composition may be a vaccine composition for administration to a human in need thereof, especially children. Said composition may be used for the treatment against LASV infection. Said composition may be used for the protection against LASV. Thus, the composition may be an immunogenic or antigenic composition for the protective or prophylactic treatment against a LASV infection. In particular, the active ingredients or active principles within the composition comprise recombinant MeV-LASV particles, said recombinant MeV-LASV particles being rescued from a transfer plasmid vector according to the invention and being optionally associated with VLPs comprising the Z protein and optionally other protein(s) of LASV, or antigenic fragment(s) thereof. In the context of the invention, the terms “associated” or “in association” refer to the presence, in a single composition, of both MeV-LASV recombinant viral particles and LASV polypeptides or proteins, in particular as VLPs, usually as physically separate entities. In a particular embodiment of the invention, the composition is a vaccine.

The invention also concerns the recombinant MeV-LASV infectious replicating virus particles in association with LASV polypeptide(s) or protein(s), or antigenic fragment(s) thereof, possibly associated LASV VLPs, or any composition

according to the invention, for the use in the treatment or the prevention of an infection by Lassa virus in a subject, in particular a human subject, in particular a child.

The invention also concerns recombinant MeV-LASV infectious, replicative virus and associated LASV polypeptide(s) or protein(s), or antigenic fragment(s) thereof, and potentially associated LASV VLPs for use in an administration scheme and according to a dosage regime that elicits an immune response, advantageously a protective immune response, against LASV virus infection or induced disease, in particular in a human subject, in particular a child.

In a particular embodiment of the invention, the composition or the use of the composition is able to elicit immunization of a subject, in particular a human subject, in particular a child, after a single injection. In other words, the composition or the use of the composition may require a single administration of a selected dose of the recombinant MeV-LASV infectious replicative virus. Alternatively, it may require multiple doses administration in a prime-boost regimen. Priming and boosting may be achieved with identical active ingredients consisting of recombinant MeV-LASV infectious, replicative virus and associated LASV polypeptide(s) and protein(s), or antigenic fragment(s) thereof, and/or LASV VLPs.

The invention also concerns an assembly of different active ingredients including as one of these ingredients recombinant MeV-LASV infectious, replicative virus and associated LASV polypeptide(s) or protein(s), and/or LASV VLPs. The assembly of active ingredients is advantageously for use in immunization of a host, in particular a human host.

The inventors have shown that administration of recombinant MeV-LASV infectious, replicative virus elicits an immune response and especially elicits production of neutralizing antibodies against LASV-related polypeptides. Accordingly, it has been shown that administration of the active ingredients according to the invention elicits immunization of the host. The vaccine according

to the invention is safe, leads to immune answer within the host, which encompasses especially CD4+ and CD8+ T cell responses. As shown in the examples, the vaccine according to the invention induces antigen-specific T cell responses. It has also been shown that immunized monkey hosts survive lethal dose challenge of LASV.

After immunization of a host, and LASV challenge, the level of liver enzymes (ALT and AST), lactate deshydrogenase (LDH), C-reactive protein (CRP) and albumin remained normal or slightly increases in immunized monkey hosts, while these levels increase several folds in non-immunized hosts.

The composition according to the invention is able to elicit production of recombinant LASV-specific immunoglobulins, especially IgM and IgG, and neutralizing antibodies. The composition according to the invention is a safe vaccine, immunogenic and efficacious in a host. The compositions and their use confer at least T cell response and confer immunity against a Lassa virus infection in a vaccinated host.

The composition according to the invention may also be able to elicit production of MeV-specific immunoglobulins, especially IgM and IgG, and neutralizing antibodies. The composition according to the invention is a safe vaccine, immunogenic and efficacious in a host. The compositions and their use confer at least T cell response and may confer immunity against a Measles virus infection in a vaccinated host.

The composition according to the invention also concerns recombinant MeV-LASV infectious, replicative virus and associated LASV polypeptide(s) or protein(s), or antigenic fragment(s) thereof, and potentially associated LASV VLPs for use in an administration scheme and according to a dosage regime that elicits an immune response, advantageously a protective immune response, against measles virus infection or induced disease, in particular in a human subject, in particular a child.

The invention also concerns the recombinant MeV-LASV infectious replicating virus particles in association with LASV polypeptide(s) or protein(s), or antigenic fragment(s) thereof, and/or LASV VLPs, or any composition according to the invention, for the use in the treatment or the prevention of an infection by measles virus in a subject, in particular a human subject, in particular a child.

The invention also concerns a heterologous polynucleotide comprising any one of the codon-optimized sequence encoding the GPC protein, the Z protein, the NP protein and/or the mNP protein. Thus, the invention also concerns the codon-optimized polynucleotide comprising or consisting of SEQ ID No: 2; SEQ ID No: 4, SEQ ID No: 6 and/or SEQ ID No: 8.

DESCRIPTION OF THE FIGURES

Some of the figures, to which the present application refers, are in color. The application as filed contains the color print-out of the figures, which can therefore be accessed by inspection of the file of the application at the patent office.

Figure 1. **Schematic representation of nucleic acid constructs**. **A**: MeV vector. **B**: nucleic acid constructs according to the invention. MeV genes are indicated in grey and LASV genes are indicated in green, blue and red. For the MV genes: N (nucleoprotein); P/V/C (phosphoprotein and V/C proteins); M (matrix); F (Fusion protein); H (hemagglutinin): L (polymerase). For the LASV genes: NP (nucleoprotein); NP_{ExoN} (also referenced NP_{KO} on some figures; mutated sequence encoding a mutated NP with its exonuclease activity knocked down); GPC (glycoprotein precursor); Z (zinc-binding protein). ATUs are indicated by the black arrows. ATU1 is localized on the left, upstream the N gene of MeV while ATU2 is localized centrally, between P and M MeV genes.

Figure 2. **Growth kinetics of viruses on Vero E6 cells.** MeV-GFP corresponds to a construct wherein a polynucleotide encoding a Green Fluorescent Protein has been inserted within ATU2. MeV-GPC_{LASV} corresponds to a construct wherein a polynucleotide encoding the GPC protein of LASV has been inserted

within ATU2. MeV-NP+GPC_{LASV} corresponds to a construct wherein genes encoding the GPC protein and the NP protein of LASV has been inserted within ATU2. MeV-NP_{ExoN}+GPC_{LASV} corresponds to a construct wherein polynucleotide encoding the GPC protein and a mutated NP protein (exonuclease activity knocked down) of LASV has been inserted within ATU2. MeV-Z+GPC_{LASV} corresponds to a construct wherein a polynucleotide encoding the GPC protein has been inserted within ATU2, and wherein a polynucleotide encoding the Z protein has been inserted within ATU1. Titers obtained in typical experiments, measured by $TCID_{50}$ from 3 independent experiments. Means and standard errors are represented.

Figure 3. Expression of LASV proteins (GPC, NP and Z) **and MeV protein** (F) **in infected Vero E6 cells and in the supernatants of infected Vero E6 cells**. The effect of each construct was assessed by Western blot as detailed in the material and method. NI: non-infected cells. ns: non specific.

Figure 4. MeV-GFP entry and replication in immune antigen presenting cells derived from human peripheral blood mononuclear cells.

Figure 5. Expression of type I IFN in human primary macrophages infected with different MeV-LASV vectors. Quantitative RNA analysis by qPCR analyses of type I IFN response (quantitative expression of IFNa1, IFNa2 and IFNb). Expression 24h post-infection. All results are normalized to GAPDH gene and expressed as fold induction relative to GAPDH.

Figure 6. Cell surface expression of cluster of differentiation markers CD80, CD86 and CD83 CD40 in macrophages infected with different MeV-LASV vectors. Flow cytometry for the cell surface expression of co-activation molecules 48h post-infection.

Figure 7. Expression of type I IFN in human primary dendritic cells infected with different MeV-LASV vectors. Quantitative RNA analysis by qPCR analyses of type I IFN responses (quantitative expression of IFNa1, IFNa2 and IFNb). Expression 24h post-infection. All results are normalized to GAPDH gene and expressed as fold induction relative to GAPDH.

Figure 8. Cell surface expression of cluster of differentiation markers CD80, CD86, CD83 and CD40 in human primary dendritic cells infected with

different MeV-LASV vectors. Flow cytometry for the cell surface expression of co-activation molecules 48h post-infection.

Figure 9. Body temperature in cynomolgus monkeys (*Macaca fascicularis*) during a 30-day period post immunization. 3, 4 and 4 monkeys were subcutaneously immunized with 2.10^6 Tissue culture Infective Dose 50 ($TCID_{50}$) of respectively a recombinant MeV strain Schwarz vaccine, a recombinant MeV-NP_{ExoN}-GPC vaccine and a recombinant Z-MeV-GPC vaccine.

Figure 10. LASV antigens-specific CD4 and CD8 T cells responses in vaccinated cynomolgus monkeys (*Macaca fascicularis*). Flow cytometry after stimulation of whole blood by overlapping peptides specific to GPC, NP and Z.

Figure 11. Clinical scores in cynomolgus monkeys (*Macaca fascicularis*) after challenge with a lethal dose of LASV strain Josiah. Clinical score is based on body temperature, body weight, capacity to feed and hydrate normally, behavior, clinical signs. A score of 15 is the endpoint for killing. Lethal dose of LASV strain consists in 1.500 FFU of LASV strain Josiah subcutaneously injected to the animals.

Figure 12. Body temperature in cynomolgus monkeys (*Macaca fascicularis*) challenged with a lethal dose of LASV strain Josiah.

Figure 13. Liver enzymes (AST and ALT) levels in plasma of immunized cynomolgus monkeys.

Figure 14. Plasma level of LDH (A), CRP (B) and albumin (C) in cynomolgus monkeys (*Macaca fascicularis*) challenged with a lethal dose of LASV strain Josiah.

Figure 15. Viremia (RNA(A) and Titer (B)) in cynomolgus monkeys challenged with a lethal dose of LASV strain Josiah. RNA quantification by qPCR. Titration according to known method in the art.

Figure 16. Viral RNA quantification in the nasal (A) and oral secretions (B) and in the urine (C) of cynomolgus monkeys challenged with a lethal dose of LASV strain Josiah. RNA quantification by qPCR.

Figure 17. LASV RNA levels detected in organs of challenged cynomolgus monkeys previously immunized with different MeV-LASV.

Figure 18. LASV infectious titers detected in organs of challenged cynomolgus monkeys previously immunized with different MeV-LASV.

Figure 19. IgM and IgG responses against LASV in cynomolgus monkeys challenged with a lethal dose of LASV strain Josiah. A. IgM LASV specific. B: IgG LASV specific. Immunoglobulin levels measured by ELISA. Optical density calculated according to the absorbance at 450 nM.

Figure 20. LASV GP- and NP- specific CD8+ and CD4+ T cell responses after immunization. The percentage of CD8+ and CD4+ T cells that produced IFNg, TNFa and/or IL-2 after stimulation with overlapping peptides covering the whole LASV GP and NP have been determined using flow cytometry.

Figure 21. LASV antigens-specific CD4 and CD8 T cell responses of immunized cynomolgus monkeys challenged with a lethal dose of LASV strain Josiah. Flow cytometry after stimulation of whole blood by overlapping peptides specific to GPC and NP.

Figure 22. Proliferation and activation of CD4 and CD8 T cells in of cynomolgus monkeys challenged with a lethal dose of LASV strain Josiah. CD8 proliferation assessed by Ki67 staining (A). CD4 (B) and CD8 (C) Activation assessed by quantification of granzyme B expression.

Figure 23. LASV GP- and NP-specific CD8+ T cell responses after LASV challenge. The percentage of CD8+ T cells that produced IFNg, TNFa and/or IL-2 after LASV challenge with overlapping peptides covering the whole LASV GP (23A) and NP (23B) have been determined with flow cytometry. The proportion of different sub-populations of responding T cells is presented using pie chart.

Figure 24. LASV GP- and NP-specific CD4+ T cell responses after LASV challenge. The percentage of CD4+ T cells that produced IFNg, TNFa and/or IL-2 after LASV challenge with overlapping peptides covering the whole LASV GP (23A) and NP (23B) have been determined with flow cytometry. The proportion of different sub-populations of responding T cells is presented using pie chart.

Figure 25. KEGG pathway analysis performed on the transcriptomic data obtained from cynomolgus monkeys PBMC collected at different time points post-immunization with MeV-NP$_{ExoN}$-GPC$_{LASV}$.

Figure 26. KEGG pathway analysis performed on the transcriptomic data obtained from cynomolgus monkeys PBMC collected at different time points post-immunization with MeV-Z+GPC$_{LASV}$.

Figure 27. Quantification of cytokines in the plasma of immunized monkeys after LASV challenge. Different cytokines have been quantified in the plasma of MeV-, MeV-NP_{ExoN}-GPC_{LASV}, and MeV-Z+GPC_{LASV} immunized cynomolgus monkeys after LASV challenge. Significant differences ($p<0.05$) between different conditions are indicated: n-c (MeV-NP_{ExoN}-GPC_{LASV} and MeV), n-z (MeV-NP_{ExoN}-GPC_{LASV} and MeV-Z+GPC_{LASV}) and n-cz (MeV-NP_{ExoN}-GPC_{LASV} and MeV; MeV-NP_{ExoN}-GPC_{LASV} and MeV-Z+GPC_{LASV}).

Figure 28. IgM and IgG responses against MeV in cynomolgus monkeys challenged with a lethal dose of LASV strain Josiah. A: IgM MeV specific. B: IgG MeV specific. IgG and IgM MeV-specific were not measures at days 7 and 14 for the monkeys immunized with the MeV construct.

Figure 29. Determination of the Exonuclease activity of native and mutated NP protein. Fold induction of Luciferase activity virus-induced and immunostimulatory RNAs-induced interferon-beta activation. CT: control. NP_{LASV}: native NP protein. $NP_{ExoNLASV:}$ mutated NP protein of SEQ ID No: 5. SeV : Sendai virus at moi=1.

Figure 30. Analysis of MeV-NP_{ExoN}-GPC_{LASV} tropism. CHO cell lines were infected with either a Mopeia virus pseudotyped with LASV GPC or with MeV-NP_{ExoN}-GPC_{LASV}. Expression of GPC was analyzed by staining with an anti-GP1 antibody. The nuclei are in blue, while the anti-GP1 stained is in green.

Figure 31. Schematic representation of transfer vector plasmid according to a first embodiment of the invention. The transfer vector has the sequence of SEQ ID No: 9. The measles gene encoding the N protein is localized between nucleotides 189 and 1767. The measles gene encoding the P protein is localized between nucleotides 1889 and 3412. The codon-optimized heterologous polynucleotide of SEQ ID No: 2 encoding the GPC is localized between nucleotides 3532 and 5007. ATU2 is localized between nucleotides 3487 and 5071 minus the heterologous polynucleotide insert. The measles gene encoding the M protein is localized between nucleotides 5104 and 6111.

Figure 32. Schematic representation of transfer vector plasmid according to a second embodiment of the invention. The transfer vector has the sequence of SEQ ID No: 10. The measles gene encoding the N protein is localized between nucleotides 190 and 1767. The measles gene encoding the P

protein is localized between nucleotides 1889 and 3412. The codon-optimized heterologous polynucleotide of SEQ ID No: 4 encoding the NP protein is localized between nucleotides 3532 and 5241. The codon-optimized heterologous polynucleotide of SEQ ID No:2 encoding the GPC is localized between nucleotides 5386 and 6861. A linker sequence comprising regulatory sequence of the measles virus is localized between nucleotides 5242 and 5385. ATU2 is localized between nucleotides 3487 and 6925 minus the heterologous polynucleotide insert and the linker sequence. The measles gene encoding the M protein is localized between nucleotides 6958 and 7965.

Figure 33. Schematic representation of transfer vector plasmid according to a third embodiment of the invention. The transfer vector has the sequence of SEQ ID No: 11. The measles gene encoding the N protein is localized between nucleotides between 190 and 1767. The measles gene encoding the P protein is localized between nucleotides 1889 and 3412. The codon-optimized heterologous polynucleotide of SEQ ID No: 6 encoding the mutated NP protein is localized between nucleotides 3532 and 5241. The codon-optimized heterologous polynucleotide of SEQ ID No:2 encoding the GPC is localized between nucleotides 5386 and 6861. A linker sequence comprising regulatory sequence of the measles virus is localized between nucleotides 5242 and 5385. ATU2 is localized between nucleotides 3487 and 6925 minus the heterologous polynucleotide insert and the linker sequence. The measles gene encoding the M protein is localized between nucleotides 6958 and 7965.

Figure 34. Schematic representation of transfer vector plasmid according to a fourth embodiment of the invention. The transfer vector has the sequence of SEQ ID No: 12. The codon-optimized heterologous polynucleotide of SEQ ID No: 8 encoding the Z protein is localized between nucleotides 193 and 504. The measles gene encoding the N protein is localized between nucleotides 646 and 2223. The measles gene encoding the P protein is localized between nucleotides 2345 and 3868. The codon-optimized heterologous polynucleotide of SEQ ID No: 2 encoding the GPC is localized between nucleotides 3988 and 5463. The measles gene encoding the M protein is localized between nucleotides 5560 and 6567.

Figure 35. Schematic representation of transfer vector plasmid according to a fifth embodiment of the invention. The transfer vector has the sequence of SEQ ID No: 13. The codon-optimized heterologous polynucleotide of SEQ ID No: 8 encoding the Z protein is localized between nucleotides 193 and 504. The measles gene encoding the N protein is localized between nucleotides 646 and 2223. The measles gene encoding the P protein is localized between nucleotides 2345 and 3868. The codon-optimized heterologous polynucleotide of SEQ ID No: 4 encoding the NP protein is localized between nucleotides 3988 and 5697. The codon-optimized heterologous polynucleotide of SEQ ID No:2 encoding the GPC is localized between nucleotides 5842 and 7317 The measles gene encoding the M protein is localized between nucleotides 7414 and 8421.

Figure 36. Schematic representation of transfer vector plasmid according to a sixth embodiment of the invention. The transfer vector has the sequence of SEQ ID No: 14. The codon-optimized heterologous polynucleotide of SEQ ID No: 8 encoding the Z protein is localized between nucleotides 193 and 504. The measles gene encoding the N protein is localized between nucleotides 646 and 2223. The measles gene encoding the P protein is localized between nucleotides 2345 and 3868. The codon-optimized heterologous polynucleotide of SEQ ID No: 6 encoding the mutated NP protein is localized between nucleotides 3988 and 5697. The codon-optimized heterologous polynucleotide of SEQ ID No:2 encoding the GPC is localized between nucleotides 5842 and 7317 The measles gene encoding the M protein is localized between nucleotides 7414 and 8421.

EXAMPLES

Materials and methods

Cells and viruses

293T7/N/P cells expressing stably the T7 polymerase and the measles N and P proteins were used to rescue recombinant measles viruses and were maintained as described before {Combredet, 2003 #76}. Vero NK cells were grown in Glutamax Dulbecco Modified Eagle's Medium (DMEM, Life Technologies) supplemented with 5% FCS, and 0.5% Penicillin-Streptomycin. Blood samples

were obtained from the Etablissement Français du Sang (EFS, Lyon, France). Mononuclear cells were purified by Ficoll density gradient centrifugation (GE Healthcare). Monocytes were first separated from peripheral blood mononuclear cells by centrifugation on a cushion of 50% Percoll (GE Healthcare, Velizy, France) in PBS and then purified using the Monocyte isolation kit II according to the manufacturer's instructions (Miltenyi Biotec, Paris, France). Macrophages were obtained by incubating monocytes for 6 days in RPMI, 10% SVF, 10% autologous serum supplemented with 50 ng/mL of M-CSF. M-CSF was added every 2 days and 40% of the culture medium was replaced.

Plasmid constructs

Codon-optimized ORF of LASV GPC, NP and Z (LASV strain Josiah) were cloned in the pTM1-MVSchwarz vector in additional transcriptional units (ATU) placed upstream of Nucleoprotein (N) (ATU1 for Z) or between the phosphoprotein (P) and the matrix (M) genes of the Schwarz MV genome (ATU2, GPC alone or NP+GPC) like previously described (Combredet, C., et al., A molecularly cloned Schwarz strain of measles virus vaccine induces strong immune responses in macaques and transgenic mice. J Virol, 2003. 77(21): p. 11546-54). All plasmid constructs were verified by sequencing.

Western blot and antibodies

Vero NK cells infected with recombinant MeV-GFP, MeV- GPC_{LASV}, MeV-NP+GPC_{LASV}, MeV-NP_{ExoN}+GPC_{LASV} or MeV-Z+GPC_{LASV} were lysed in Co-IP buffer and cleared by centrifugation. Lysates and culture supernatants were then separated on 4-12% precast gels (Biorad) under denaturing conditions and transferred to PVDF membrane. Membranes were stained with primary antibodies to GP1 (in house mouse monoclonal production), NP (mouse anti-LASV serum), Z (in house rabbit polyclonal production) or F (rabbit polyclonal Fcyt, a kind gift of R. Cattaneo). Cell lysates were also stained with an anti-actin antibody coupled to the horseradish peroxidase (HRP). After staining with secondary antibodies coupled to HRP, membranes were revealed using West Dura substrate (Pierce) and photographed using a LAS4000 imager (GE Healthcare).

Virus rescue and titration

Recombinant measles viruses expressing LASV antigens were rescued as previously described (Combredet, 2003; Radecke, F., *et al.*, Rescue of measles viruses from cloned DNA. Embo J, 1995. 14(23): p. 5773-84; WO2008/078198). Briefly, 293T7/N/P cells were transfected with plasmids encoding the measles L polymerase and the antigenomic segment of the desired MeV vector. Clonal syncytia were picked and used to infect Vero NK cells in 6-well plates. When syncytia has reached about 50% of the well superficy, cells were detached and overlaid on Vero NK cells in 10 cm dishes to produce passage 1 (P1) stocks. To prepare higher passage virus stocks, Vero NK cells were infected at a multiplicity of infection (MOI) of 0.01 and then incubated at 32°C for 2 to 3 days. To harvest virus, cells were scraped into Opti-MEM I reduced-serum medium and freeze-thawed twice. Titers were determined by 50% tissue culture infective dose (TCID50) titration on Vero NK cells.

Quantitative RNA analysis

For RT-qPCR experiments, total RNA was isolated from mock or infected cells using the Rneasy Mini Kit (Qiagen, Courtaboeuf, France), according to the manufacturer's instructions, and a supplementary DNase step added using the Turbo DNA free kit Ambion (Thermo Fisher Scientific). Synthesis of cDNA was performed using SuperScript III and amplification was performed using the Gene Expression Master Mix kit (Applied Biosystems, Thermo Fisher Scientific). For type I IFN, the primer/probe mix was developed in house. Runs of qPCR assays were performed in a LightCycler 480 (Roche Diagnostics, Meylan, France). The expression of all genes was standardized to that of the GAPDH gene, and expressed as fold induction relative to GAPDH. For viral RNA quantification, an RNA probe of the 771-934pb region of the NP ORF was cloned into the pGEM vector (Promega) to generate T7 polymerase driven transcripts. The RNA probe was DNAse treated, purified, and quantified (Dropsense96, Trinean, Gent, Belgium). Quantitative PCR for viral RNA was performed with the EuroBioGreen qPCR Mix Lo-ROX (Eurobio, Les Ulis, France), using LASV specific primers.

Flow Cytometry for MP activation, T cell activation and proliferation

Mock and MOI 1-infected MP were detached 48 h after infection, saturated with human IgG and surface stained with antibodies to CD40, CD83, CD80, and CD86 (BD Biosciences, Le-Pont-de-Claix, France) before final fixation in PBS/1% PFA. LASV antigen-specific T cells were analysed from fresh whole blood. Cells were incubated with a pool of GPC, NP or Z overlapping peptides in the presence of CD28 and CD49d antibodies (2 µg/ml) and Brefeldin A (10 µg/ml) for 6h at 37°C. SEA (1µg/ml) or PBS were respectively used as positive or negative control of activation. Peptides are 15-mer amino acids long (1 µg/ml each) with an overlap of 11 residues and spanned the complete GPC, NP or Z ORF of LASV strain Josiah. PBS-EDTA 20mM was added to samples before cell-surface staining for CD3, CD4 and CD8 (BD Biosciences). Red blood cells were then lysed using PharmLyse (BD Biosciences). Cells were then fixed and permeabilized for intracellular staining with antibodies to IFNγ (Biolegend). For proliferation and activation, wells were stained using antibodies to Ki67 or Granzyme B. Cells were analysed by flow cytometry using an LSR Fortessa cytometer (BD Biosciences) or a 10-color Gallios cytometer (Beckman Coulter). Data were analysed using Kaluza software (Beckman Coulter).

Cynomolgus monkey challenge with LASV

Groups of 4 male cynomolgus monkeys (Macaca Fascicularis, 32-39 month-old, 3-4 kg) were immunized in A2 facilities (SILABE, France) by subcutaneous injection of 2.106 TCID50 of MeV-NP_{ExoN}+GPC_{LASV} or MeV-Z+GPC_{LASV}, respectively. Another control group of 3 monkeys was immunized with the MeV vaccine strain Schwarz. Blood draws, oral and nasal swabs and urine sampling were performed every 2-3 days during the first two weeks then once a week up to day 37 in order to assess vaccine replication and shedding, IgM and IgG responses and T cell responses against LASV GPC, NP or Z. After 37 days, monkeys were transported to BSL-4 facilities (Laboratoire P4-Inserm Jean Mérieux) where they were challenged subcutaneously using 1,500 FFU of LASV strain Josiah. Animals were followed for clinical signs of the disease and were euthanized according to scoring made based on body temperature, body weight, feeding, hydrating, behaviour and clinical signs. Experimentation endpoint was

placed at day 28 post challenge and all animals that had survived to this point were euthanized according to validated experimental procedures. Blood draws, oral and nasal swabs and urine sampling were performed every 2-3 days during the first two weeks then once a week up to day 28 in order to assess LASV virus replication and shedding, IgM and IgG responses and T cell responses against LASV GPC, NP or Z. This study has been approved by the Comité Régional d'Ethique en Matière d'Expérimentation Animale de Strasbourg (APAFIS#6543-20160826144775) and by the Comité Régional d'Ethique pour l'Expérimentation Animale Rhône Alpes (CECCAPP 20161110143954).

Determination of the Exonuclease activity of native and mutated NP protein (Fig. 29):

293T cells were cotransfected using calcium phosphate with 100 ng of a vector that expresses the firefly luciferase (Fluc) reporter gene from a known functional promoter sequence of the IFN-beta gene (pIFNbeta-LUC), variable amounts of either native (wild type) or mutant LASV NP vectors, and 50 ng of a β-gal-expressing plasmid for transfection normalization. At 24 h post- transfection, cells were infected with Sendai virus (at moi = 1) in order to induce IFN-β expression. At 24 hpi, cell lysates were prepared for luciferase and β-gal assays. Fluc activities were normalized by the β-gal values. To determine whether NP has an exonuclease activity or not, its effect on the suppression of the immunostimulatory RNAs-induced IFN production is analysed, HEK293 cells were transfected with pIFNbeta-LUC, variable amounts of either native (WT) or mutant LASV NP vectors, and a beta-gal-expressing plasmid for transfection normalization. 18 h later, cells were transfected with either 1 µg of Poly(I:C) or 250 ng of Pichinde virion RNAs by lipofectamine 2000. Luciferase activity was determined at 18 h after the immunostimulatory RNA transfection and normalized by the beta-gal activity. A mutated NP protein with its exonuclease activity knocked down does not suppress the immunostimulatory RNAs-induced IFN production.

Example 1 – Generation of recombinant MeV viruses expressing LASV antigens

In order to determine what is the best combination of LASV antigens to introduce in the MeV vector to get the best immunogenicity, we have generated several MeV/LASV vaccine candidates using the Schwarz MeV vaccine platform (Fig. 1A) expressing GPC alone or in combination with NP (mutated or not in the exonuclease domain to abrogate the immunosuppressive function contained in the LASV NP) or GPC and Z in order to produce antigenic LASV virus-like particles (VLPs) in vivo. We also made constructs expressing Z, GPC and NP mutated or not in the exonuclease domain. The GPC and NP genes were cloned between the MeV P and M genes in the additional transcriptional unit 2 (ATU2). The Z gene was cloned upstream of the N gene in the ATU1 (Fig.1B).

All viruses were rescued and grew to similar titers on Vero E6 cells than a control MeV-GFP expressing GFP from ATU2 (Fig. 2), except for the MeV/LASV-Z+GPC that was attenuated by about a log compared to other constructs. Expression of the different LASV antigens was controlled by western blot using specific antibodies against LASV GPC, NP or Z, or against the measles fusion protein F (Fig. 3). Expression of GPC was detected in MeV-GPC_{LASV}, MeV-NP+GPC_{LASV}, MeV-NP_{ExoN}+GPC_{LASV} and MeV-Z+GPC_{LASV} infected Vero E6 cells; expression of NP in MeV-NP+GPC_{LASV}, MeV-NP_{ExoN}+GPC_{LASV} and MeV-Z+GPC_{LASV} infected Vero E6 cells; expression of Z only in MeV-Z+GPC_{LASV} infected cells. MeV F expression was detected in all MeV infected cells. As expected, GPC was also detected in the supernatants of MeV-Z+GPC_{LASV} infected cells along with Z, supporting the release of GPC along with Z under the form of VLPs. All vectors were passaged 10 times without loss of LASV antigens expression.

Example 2 – immunogenicity of MeV viruses expressing LASV antigens in human primary antigen presenting cells

To characterize the immunogenic properties of the different MeV vectors in human immune cells, we infected monocyte derived macrophages and dendritic cells. MeV enters and replicate in these cells, as shown by the expression of GFP

on figure 4. However, viruses did not replicate efficiently in these cells as infectious titers were barely detectable at day 1 post infection and did not increase over time, likely due to the induction of an antiviral innate response.

We analysed the immune responses of macrophages and dendritic cells to the different recombinant viruses by combining flow cytometry analyses of activation markers and qPCR analyses of the type 1 IFN response. We analysed the type I IFN responses induced by the different vectors by qPCR at 24 hrs post infection (Fig. 5). In macrophages, MeV-GPC$_{LASV}$ and MeV-Z+GPC$_{LASV}$ induced the same levels of IFN alpha-1, alpha-2 and beta than the control MeV-GFP. However, addition of the LASV NP reduced by almost 3 logs the induction of type I IFN (MeV-NP+GPC$_{LASV}$) but mutation of the ExoN domain of LASV NP (MeV-NP$_{ExoN}$+GPC$_{LASV}$) restored the induction of type I IFN to levels comparable to MeV-GFP and -GPC$_{LASV}$. This results demonstrate that the LASV NP can control the induction of type I IFN through its ExoN activity, likely by digesting dsRNA molecules expressed during MeV replication (Son, 2015). We then looked at the induction of co-activation molecules by the different vectors at 48 hrs post infection in macrophages (Fig. 6). Importantly, cell surface expression of co-activation molecules is critical to activate the T cell responses. As shown on figure 6, all vectors induced strong cell surface expression of CD80, CD86 and CD83. Notably, expression was reduced in MeV-NP+GPC$_{LASV}$ infected macrophages compared the expression in macrophages infected by the other vectors but was restored when the ExoN domain of LASV NP was mutated.

Similar experiments were performed on dendritic cells (Fig. 7 and 8). As observed in macrophages, MeV-GPC$_{LASV}$ and MeV-Z+GPC$_{LASV}$ induced the same levels of IFN alpha-1, alpha-2 and beta than the control MeV-GFP (Fig. 7). Addition of LASV NP also reduced the induction of type I IFN, but mutation of the ExoN domain of LASV NP restored the induction of type I IFN to levels comparable to MeV-GFP, MeV-Z+GPC$_{LASV}$ and MeV-GPC$_{LASV}$. The expression of the co-activation molecules CD80, CD86, CD83 and CD40 were also similarly induced in dendritic cells infected with MeV-GFP, MeV-Z+GPC$_{LASV}$ and MeV-GPC$_{LASV}$ (Fig. 8).

The vaccine strains of MeV-LASV induce a type I IFN response and cell surface expression of co-activation molecules; the presence of wild type NP strongly reduces the ability of the vaccine to induce these effects, but mutation within the ExoN domain restore the ability of the vaccine to induce these effects.

Example 3 – Safety, immunogenicity and efficacy of two vaccines in cynomolgus monkeys

Based on the results obtained in human macrophages, we decided to test two vaccine candidates in cynomolgus monkeys, the gold standard model to study LASV pathogenesis. Three control animals were immunized subcutaneously with 2.106 TCID50 of a recombinant MeV strain Schwarz vaccine and two groups of 4 animals were immunized subcutaneously with 2.106 TCID50 of MeV-NP_{ExoN}+GPC_{LASV} and MeV-Z+GPC_{LASV}, respectively. The health of the animals was then followed for 37 days post immunization (body temperature, body weight, respiratory rate) and no adverse effects were noted. Notably, the body temperature of the animals, continuously monitored thanks to intraperitoneal devices, was not altered by the immunization (Fig. 9).

We also assessed the viremia in immunized animals every 2-3 days during the two weeks following immunization then once a week and could not detect any trace of viral RNA, neither in plasma nor among PBMC. Similarly, we could not detect any viral RNA in the nasal and oral secretions or in the urine of vaccinated animals. Thus, it appears that the vaccine candidates are safe in monkeys and are not shed at any moment post immunization.

In order to assess the immunogenicity of the vectors, we performed ELISA to detect LASV-specific IgM and IgG. We could not detect specific IgM and IgG in MeV-Z+GPC_{LASV} immunized animals and we only detected low levels of LASV-specific IgG in 3 out of 4 MeV-NP_{ExoN}+GPC_{LASV} vaccinated animals at day 37 post immunization. In addition, one MeV-NP_{ExoN}+GPC_{LASV} vaccinated animal had neutralizing antibodies as demonstrated by plaque reduction neutralization assay

(1:100 titer). We also assessed the LASV-specific T cell responses by flow cytometry after stimulation of whole blood by overlapping peptides specific to GPC, NP or Z. In MeV-NP_{ExoN}+GPC_{LASV} vaccinated animals, we detected both CD4 and CD8 T cell responses against GP starting at day 7 and decreasing by day 14 post immunization (Fig. 10A and 10C, orange bars). In these animals, we also detected both CD4 and CD8 T cell responses against NP between days 10 and day 21 post immunization (Fig. 10B and 10D, orange bars). In MeV-Z+GPC_{LASV} immunized animals, the CD4 T cell response against GPC was delayed compared to MeV-NP_{ExoN}+GPC_{LASV} vaccinated animals, starting by day 10 post immunization but lasting until day 21 post inoculation (Fig. 10A, green bars). GPC-specific CD8 T cell responses started at day 7 at low levels but peaked at day 21 post immunization (Fig 10C, green bars). Both CD4 and CD8 Z-specific T cell responses were also detected starting at day 7 and until day 30, with a peak at day 21 post immunization (Fig. 10B and 10D, green bars). Importantly, no T cell responses against LASV antigens were detected in MeV-vaccinated control animals. Thus, both vaccine candidates induce LASV antigen-specific T cell responses, with MeV-NP_{ExoN}+GPC_{LASV} inducing earlier responses than MeV-Z+GPC_{LASV}.

Example 4 – Efficacy of the vaccines

In order to test the efficacy of the vaccine candidates, immunized animals were challenged 37 days post immunization with a lethal dose (1,500 ffu, subcutaneous) of LASV strain Josiah. Animals were then monitored for up to 30 days and were attributed clinical scores based on their body temperature, body weight, capacity to feed and hydrate normally, behaviour, clinical signs, with a score of 15 being the endpoint for killing. The three control animals had scores increasing from day 3 and had to be euthanized respectively at day 12, 14 and 15 post challenge (Fig. 11A).

On the contrary, all vaccinated animals survived the LASV infection but the clinical outcomes were different depending on the vaccine. Indeed, MeV-NP_{ExoN}+GPC_{LASV} vaccinated animals had a small increase in clinical score by day 5 (max score of 3, Fig; 11B), mainly due to an elevated temperature (see Figure

12, center graph). On the contrary, MeV-Z+GPC$_{LASV}$ immunized animals experienced severe symptoms such as high fever between day 3 and day 12 (see figure 12, lower graph) and while 2 animals totally recovered by day 12, 2 other animals had difficulties feeding and hydrating, showed prostration and one animal had balance issues and lost more than 7.5% of his body weight by day 30, reaching a score of 14 (Fig. 11C).

We also followed several biological parameters in plasma over the course of the infection, such as liver enzymes levels (ALT and AST), lactate deshydrogenase (LDH), C-reactive protein (CRP) and albumin, among other parameters. In control animals, the levels of liver enzymes were increasing continuously starting at day 6 and until the death of the animals (Fig. 13, left panels). On the contrary, liver enzyme levels remained normal at any time in MeV-NP$_{ExoN}$+GPC$_{LASV}$ vaccinated animals (Fig. 13, center panels) and only slightly increased in MeV-Z+GPC$_{LASV}$ immunized animals between day 6 and 15 (Fig. 13, right panels).

The plasma levels of LDH are a marker of tissue damages. In control animals, LDH levels started to increase at day 6 post challenge and thus till the end of the animals (Fig. 14A, left panel). No increase was noted in MeV-NP$_{ExoN}$+GPC$_{LASV}$ vaccinated animals over the course of the infection (Fig. 14A, center panel). However, LDH levels were elevated in MeV-Z+GPC$_{LASV}$ immunized animals between day 6 and day 15, especially for two animals having LDH values similar to the control animals at day 9 (Fig. 14A, right panel), suggesting some tissular damage in these animals. The levels of CRP, a marker of inflammation, also rapidly increased in control animals until death (Fig. 14B, left panel). CRP values remained low in the MeV-NP$_{ExoN}$+GPC$_{LASV}$ group except for one animal that showed a transient increase in CRP levels by day 6 (Fig. 14B, center panel). On the contrary, all the animals from the MeV-Z+GPC$_{LASV}$ group had increased CRP levels between day 3 and day 15 (Fig. 14B, right panel). In this group, one animal showed a second wave of CRP synthesis between day 15 and day 30, suggesting that LASV virus was still replicating in this animal (Fig. 14B, right panel, light green). We also followed the plasma levels of albumin, a marker of kidney and liver dysfunction, in immunized monkeys. On control animals, albumin levels

constantly decreased starting at day 3 (Fig. 14C, left panel) while these levels remained steady in MeV-NP_{ExoN}+GPC_{LASV} immunized animals (Fig. 14C, center panel). In the MeV-Z+GPC_{LASV} group, all animals experienced a decrease in albumin plasma levels between day 3 and day 12 but these levels eventually went back to normal by day 15 (Fig. 14C, right panel).

The viremia in challenged animals was also monitored after challenge by both qRT-PCR and titration. As shown on Figure 15A, the levels of RNA levels in the blood of the infected controls increased relentlessly from day 3 to the day of killing, peaking at 109 RNA copies per mL at day 15 for one animal (left panel). Infectious titers were also detected in these animals (Fig. 15B, left panel). In MeV-NP_{ExoN}+GPC_{LASV} vaccinated animals, viral RNA was only detected at day 6 post challenge and at low levels compared to control animals (Fig. 15A, center panel) and no associated viremia was detected (Fig. 15B, center panel). The levels of viral RNA were higher in MeV-Z+GPC_{LASV} immunized animals, peaking at day 6 around 106 RNA copies per mL and decreasing until day 15 (Fig. 15A, right panel). Notably, one animal had a rebound in the number of RNA copies in the blood by day 15 (Fig. 15A, right panel, light green), which can be correlated with the rebound in CRP observed in the same animal (Fig. 14B, right panel). In addition, infectious titers were detected in all animals at day 6 and up to day 15 for the animal showing prolonged RNA levels in the blood (Fig. 15B, right panel).

In addition to viremia, we assessed the presence of viral RNA in nasal and oral swabs of challenged animals. As shown on Figure 16, levels of viral RNA peaked at day 9 in the nasal and oral secretions of control animals (Fig. 16A and B, left panels) and decreased but were still detectable at the time of death. Similarly, levels of viral RNA peaked at day 9 in these secretions for MeV-Z+GPC_{LASV} immunized animals, with a rebound for one or two animals by day 15 (Fig. 16A and B, right panels). On the contrary, only small amount of LASV RNA was detected in the nasal swab of one animal at day 3 and another animal at day 6 (Fig. 16A and B, center panels) and was not associated with the presence of infectious virus (data not shown). In addition, we followed shedding of viral RNA in the urine of challenged animals. LASV RNA levels were only detected in control

and MeV-Z+GPC$_{LASV}$ immunized animals, respectively starting at day 9 or day 15 post challenge (Fig. 16C).

The amount of LASV RNA (Fig. 17), as well as the LASV infectious titers (Fig. 18), have been analysed in different organs collected at the time of necropsy of animals immunized with MeV, MeV-NP$_{ExoN}$-GPC$_{LASV}$ and MeV-Z-GPC$_{LASV}$. All MeV control animals presented a detectable amount of LASV RNA and a high virus titer in each organ tested, except in the bladder wherein only a single animal presented a detectable amount of LASV RNA. Highest infectious titers were found in the spleen, the liver and the lung. In all animals immunized with MeV-Z+GPC$_{LASV}$, detectable amount of LASV RNA has been found in inguinal lymph node, mesenteric lymph and spleen, and detectable amount of LASV RNA has been detected in all organs, but never in a single animal, within this group. Two animals presented infectious titers of Lassa virus in the inguinal lymph node, while one animal presented infectious titer of Lassa virus in the spleen, but the other organs were free of infectious virus. In the group of animals immunized with MeV-NP$_{ExoN}$+GPC$_{LASV}$, detectable amount of LASV RNA was found in the lymphoid organs and in the lung of one to three animals, but the presence of RNA was not associated with the presence of infectious Lassa virus.

Example 5 – Immune response to LASV

In order to determine the immune response to infection, we first measured the levels of LASV-specific immunoglobulins produced after the challenge with LASV. The IgM response started at day 9 in all animals, and peaked at day 12 (Fig. 19A). Interestingly, IgM levels were higher at day 12 in animals from the control group and from the MeV-Z+GPC$_{LASV}$ group, suggesting that the level IgM response does not positively correlate with the protection but rather with the viral load. In terms of IgG responses, we noted a strong induction of LASV-IgG in all MeV-NP$_{ExoN}$+GPC$_{LASV}$ vaccinated animals by day 9, while the IgG response in MeV-Z+GPC$_{LASV}$ immunized animals only reached similar levels at day 15 (Fig. 19B). The levels of LASV-specific IgG in control animals remained very low at any time point. In addition, the seroneutralisation titers have been determined in

the plasma of immunized monkeys with MeV-NP_{ExoN}-GPC_{LASV}, MeV-Z+GPC_{LASV} and MeV at different time points post-immunization (results are illustrated in table 1). At the time of challenge (*i.e.* 37 days post-immunization, J0 in table 1), at least one animal per group had a neutralization titer of 1/100e. Fifteen days post-challenge, and until the day of necropsy, all animal vaccinated with MeV-NP_{ExoN}-GPC_{LASV} and MeV-Z+GPC_{LASV} had neutralizing antibodies between 1/100e and 1/500e, on the contrary to the animals vaccinated with MeV. Monkeys vaccinated with MeV-Z+GPC_{LASV} had the highest titer at day 30. Neutralizing antibodies have been detected in all animals except in control animals.

	MeV-NP_{ExoN}-GPC_{LASV}				MeV-Z+GPC_{LASV}			
	CDE031	CDE041	CDF053	CDI009	CDK026	CDK086	CDK106	CDG058
J0	No	No	1/100	No	1/100	No	No	No
J6	No	No	1/100	No	No	No	No	1/100
J15	1/100	1/100	1/500	1/100	1/500	1/500	1/500	1/500
J28	1/500	1/500	1/100	1/100				
J30					1/100	1/100	1/100	1/100

	MeV		
	CDH011	CDH028	CDG058
J0	No	No	No
J6	No	No	No
J15	No	No	No
J28			
J30			

Table 1: Seroneutralisation titers measured in the plasma of challenged monkeys

The induction of CD8+ and CD4+ T cells specific for LASV antigens was also monitored by quantifying the percentage of T cells producing IFNg, TNFa and/or IL-2 in response to overlapping peptides covering the whole LASV GP, NP and Z proteins (Fig. 20) after immunization. T cells failed to respond to Z peptide (data not shown). The number of cytokine-producing T cells in response to GP and NP peptides was only modestly increased in comparison with baseline levels (Day 0)

and MeV-control animals, and TNFa was the main cytokine involved in this response. Nevertheless, a non-significant increase in the percentage of GP-specific cytokine-producing CD8+ and CD4+ T cells 21 days after immunization with MeV-NP_{Exon}-GPC_{LASV}, but not after immunization with MeV-Z+GPC_{LASV}, has been observed. Furthermore, NP-specific cytokine-producing CD4+ and CD8+ T cells appeared after 14 days in immunized animals and were still present 22 days after.

Similarly, we followed the T cell response to LASV GPC, NP or Z after challenge in T cell activation assay using overlapping peptides. The CD8 and CD4 response against GPC and NP were early and robust in MeV-NP_{ExoN}+GPC_{LASV} vaccinated animals, peaking at day 9 then decreasing slowly (Fig. 21, orange bars). The CD8 and CD4 responses against GPC was delayed and less intense in MeV-Z+GPC_{LASV} immunized animals, peaking at day 12 (Fig. 21, green bars). These animals did not present a LASV-Z specific cellular response. Control animals only experienced a very weak and transient CD8 and CD4 responses against GPC and NP between day 6 and 12 (Fig. 21, red bars).

The intensity of the CD8 responses was correlated with the proliferation of these cells as assessed by a Ki67 staining (Fig. 22A), with strong proliferation of CD8 T cells in MeV-NP_{ExoN}+GPC_{LASV} immunized animals by day 9 compared to control animals and MeV-Z+GPC_{LASV} immunized animals that had a milder proliferation peaking only at day 15 (compare orange bars with red and green bars, respectively). This proliferation was also associated with cytotoxic phenotypes of the CD8 and CD4 T cell response, as shown by the early and robust expression of granzyme B in MeV-NP_{ExoN}+GPC_{LASV} immunized animals and the delayed response in control animals and MeV-Z+GPC_{LASV} immunized animals (Fig. 22B and C, compare orange bars and green bars).

The induction of LASV GP- and NP-specific T cells in animals challenged with LASV has been monitored. No production of cytokines was observed after stimulation of PBMC with LASV Z peptides after the challenge (data not shown).

The data regarding the CD8+ and CD4+ T cells producing cytokines in challenged animals are illustrated on Fig. 23 and 24 respectively. In animals immunized with MeV, no significant amount of responding cells was found. In response to the LASV GPC peptides in animals immunized with MeV-NP_{ExoN}+GPC_{LASV}, the percentage of CD8+ and CD4+ T cells producing cytokines in response to GPC peptides rose to 2% and 0.6% respectively at day 12 post challenge, and then returned to basal level by day 22. The main part of T cells produced only IFNg, but the proportion of polyfunctional CD8+ and CD4+ T cells (Pf-T) which produce at least 2 cytokines increased from day 12 to day 30. Within the CD4+ T cells group, the percentage of IFNg-producing cells decreased until day 30 post-challenge, while the opposite was observed for the Pf-T, that rose to 59%. In the animals immunized with MeV-Z+GPC_{LASV}, a moderate number of cytokine-producing CD4+ and CD8+ T cells was observed from day 15 and 12 respectively. Most T cells produced only IFNg, and the ratio of Pf-T remained around 20% for CD8+ T cells, but rose up to 40% for CD4+ T cells. At day 30 post-challenge, and for all immunized monkeys, a noticeable part of T cells produced only TNFa.

In response to the LASV NP peptide, trace amounts of cytokine-producing T cells from animals immunized with MeV were only detected 15 days post-challenge. Responding T cells from animals immunized with MeV-Z+GPC_{LASV} were detected as soon as day 6 post-challenge, with a peak response at day 12. The phenotype of T cells was various after 6 days, while at day 9, mainly TNFa-secreting T cells were present. At day 12, IFNg-producing T cells dominated whereas Pf-T proportion increased until day 30.

The total cellular RNA content of PBMC of immunized monkeys with MeV-NP_{ExoN}+GPC_{LASV} and MeV-Z+GPC_{LASV} has also been extracted at different times post-immunization to perform RNAseq and for analysing the differential expression of genes in PBMC at different time points. An enrichment analysis on differentially expressed genes has been performed using ClusterProfiler (KEGG analysis) in order to identify the pathway associated with those genes. The pathways differentially modulated through time after immunization with MeV-

NP_{ExoN}+GPC_{LASV} and MeV-Z+GPC_{LASV} are illustrated on Fig. 25 and 26 respectively. In animals immunized with MeV-NP_{ExoN}+GPC_{LASV}, pathways involved in immune responses are activated in the first week following immunization (D2 vs D0, D4 vs D0 and D7 vs D0). The activation of the hematopoietic cell lineage and the phospholipase D pathways suggests a strong proliferation of immune cells during the first four days in association with phagocytic functions (FC gamma R-mediated phagocytosis) and chemokine signalling. The increased response of Th1, Th2 and Th17 until the seventh day suggests T cells proliferation. During the second week following immunization, the pathways involved in the modulation of the immune response are activated, notably the ubiquitin-mediated proteolysis, NF-kB signalling and the Il-17 signalling pathway. Immunization with MeV-Z+GPC_{LASV} seems to induce a weaker immune response, with a weak and transient activation of Th1, Th2 and Th17 at day 4 post-immunization and a later activation of the NF-kB and IL-17 signalling pathways (at day 14) as compared to the results observed in animals immunized with MeV-NP_{ExoN}+GPC_{LASV}. Altogether, the activation of different pathways supports an active and effective cellular response to immunization.

The release of soluble mediators in plasma of animals after immunization and after LASV challenge has been observed. Among the 29 analytes quantified using Luminex assay, no difference in the levels of soluble mediator was found between animals after immunisation (data not shown). In challenged animals (Fig. 27), a transient release of IFNg was detected in the plasma of all animals, with levels peaking on day 6 and 9 post-infection in immunized animals and MeV- controls respectively. A lower concentration was nevertheless observed in the plasma of monkeys immunized with MeV-NP_{ExoN}+GPC_{LASV}. Concentrations of perforin rose until day 9 or 12 in all animals, and then decreased to a low level until day 22. Once again, the levels observed in monkeys immunized with MeV-NP_{ExoN}+GPC_{LASV} were lower. Elevated soluble CD137 (sCD137) levels were observed in monkeys immunized with MeV and MeV-Z+GPC_{LASV} 9 days after infection, while only moderate concentrations of sCD137 were observed in animals immunized with MeV-NP_{ExoN}+GPC_{LASV}. IL-6 appeared in the plasma of all animals by day 6, and was still present at day 9 in animals vaccinated with

MeV and MeV-Z+GPC$_{LASV}$, while it was not detected anymore in the plasma of animals immunized with MeV-NP$_{ExoN}$+GPC$_{LASV}$. IL-6 levels were still rising in the plasma of MeV-animals, while the increase was moderate in the plasma of animals immunized with MeV-Z+GPC$_{LASV}$. Elevated amount of IL-8 was observed in the plasma of animals immunized with MeV and MeV-Z+GPC$_{LASV}$ from day 6, whereas only low concentrations were detected between day 9 and day 12 in MeV-NP$_{ExoN}$+GPC$_{LASV}$ monkeys. IL-18 was not detected in the plasma of MeV-NP$_{ExoN}$+GPC$_{LASV}$ monkeys, while high levels and low levels was detected in animals immunized with MeV and MeV-Z+GPC$_{LASV}$ respectively. MCP1 remains at basal levels in MeV-NP$_{ExoN}$+GPC$_{LASV}$ monkeys. In contrast, high concentrations were found in MeV and MeV-Z+GPC$_{LASV}$ animals, starting from day 6 and 9 respectively. Levels of IL-10 and IL-1 receptor antagonist (IL-1RA) increased until day 9 in MeV-Z+GPC$_{LASV}$ monkeys, and then decreases to reach a low level at day 22. IL-10 and IL-1RA levels were still rising in MeV animals, while except for small amount detected after 6 days, IL-10 and IL-1RA were not released in the plasma of animals immunized with MeV-NP$_{ExoN}$+GPC$_{LASV}$.

Example 6: Immune response to MeV

The levels of MeV-specific immunoglobulins produced against MeV-specific immunoglobulins was also assessed post-challenge by ELISA (Fig. 28A: IgM and 28B: IgG). The IgM and IgG MeV-specific are produced in all animals (MeV group, MeV-NP$_{ExoN}$+GPC$_{LASV}$ group; MeV-Z+GPC$_{LASV}$ group). Similar MeV-specific IgM and IgG responses are induced by the MeV-NP$_{ExoN}$-GPC$_{LASV}$, the MeV-Z+GPC$_{LASV}$ and the MeV vaccines (Fig. 28). These animals are vaccinated MeV (Fig. 28), and animals which have been immunized with MeV-NP$_{ExoN}$+GPC$_{LASV}$ or MeV-Z+GPC$_{LASV}$ are vaccinated against LASV (See Fig. 15) and MeV (‘Fig.28).

Example 7: tropism of the vaccine strains of MeV-LASV

The tropism of MeV-LASV has been analysed. Lassa virus uses α-dystroglycan (α-DG) as a receptor. The vaccine strains of MeV use CD46, SLAM and nectin-4

as receptors. A Mopeia virus has been used as a control to analyse if the introduction of Lassa antigens into the MeV vector has an impact on the tropism of the vaccine strains of MeV. Mopeia virus is an arenavirus closely related to the Lassa virus, and uses the same receptor. A Mopeia virus pseudotyped with the Lassa virus GPC replicates in CHO-K1 cells which express α-DG and in CHO-hCD46 cells which express α-DG and the human CD46, as illustrated on Fig. 30, wherein a staining with an anti-GP1 is positive in both cell lines. On the contrary, MeV-NP_{ExoN}+GPC_{LASV} are not able to replicate in CHO-K1 cells, while it replicates in CHO-hCD46 cell line (Fig. 30, bottom pictures). Therefore, the introduction of Lassa antigens into the MeV vector does not extend the tropism the MeV.

Conclusion

To conclude, both MeV-NP_{ExoN}+GPC_{LASV} and MeV-Z+GPC_{LASV} vaccines were safe, immunogenic and efficacious in non-human primates. Both protected cynomolgus macaques against a lethal challenge with LASV strain Josiah after a single immunization. However, MeV-NP_{ExoN}+GPC_{LASV} conferred the best protection with a robust T cell response and a nearly-sterilizing immunity in all vaccinated monkeys. Thus, this vector is a candidate of choice for advance to clinical trials in humans. The immunogenicity of this vector prior challenge could certainly be improved by a prime/boost strategy. Nonetheless, we here bring the proof of principle that a single immunization could protect 100% of challenge animals. In addition, these vectors should protect monkeys against measles and could thus be used, in addition to emergency vaccine, as a bivalent vaccine in endemic countries where both LASV and MeV are major public health issues.

SEQ ID NO: 1

SEQ ID No: 1 corresponds to a recombinant GPC protein of the Lassa Virus strain Josiah encoded by the codon-optimised sequence of SEQ ID No: 2.

MGQIVTFFQEVPHVIEEVMNIVLIALSVLAVLKGLYNFATCGLVGLVTFLLLCGR
SCTTSLYKGVYELQTLELNMETLNMTMPLSCTKNNSHHYIMVGNETGLELTLT
NTSIINHKFCNLSDAHKKNLYDHALMSIISTFHLSIPNFNQYEAMSCDFNGGKIS
VQYNLSHSYAGDAANHCGTVANGVLQTFMRMAWGGSYIALDSGRGNWDCI
MTSYQYLIIQNTTWEDHCQFSRPSPIGYLGLLSQRTRDIYISRRLLGTFTWTLS
DSEGKDTPGGYCLTRWMLIEAELKCFGNTAVAKCNEKHDEEFCDMLRLFDFN

KQAIQRLKAEAQMSIQLINKAVNALINDQLIMKNHLRDIMGIPYCNYSKYWYLN
HTTTGRTSLPKCWLVSNGSYLNETHFSDDIEQQADNMITEMLQKEYMERQGK
TPLGLVDLFVFSTSFYLISIFLHLVKIPTHRHIVGKSCPKPHRLNHMGICSCGLYK
QPGVPVKWKR*

SEQ ID NO: 2

SEQ ID No: 2 corresponds to a codon-optimized nucleotide sequence encoding the GPC protein of SEQ ID No.1.

1 ATGGGCCAGA TTGTCACATT CTTTCAGGAA GTGCCACACG TCATTGAGGA GGTCATGAAC
61 ATCGTGCTGA TTGCTCTGTC AGTGCTGGCA GTGCTGAAAG GACTGTACAA CTTCGCTACC
121 TGTGGACTGG TGGGACTGGT CACATTCCTG CTGCTGTGCG GCAGAAGTTG CACTACCTCA
181 CTGTACAAAG GAGTGTACGA GCTGCAGACT CTGGAACTGA ACATGGAGAC ACTGAATATG
241 ACAATGCCTC TGAGCTGCAC CAAGAATAAT AGCCACCACT ATATCATGGT CGGGAACGAA
301 ACCGGCCTGG AACTGACCCT GACAAACACC AGCATCATTA ACCACAAGTT CTGCAATCTG
361 AGCGACGCTC ACAAGAAGAA CCTGTATGAC CACGCTCTGA TGTCCATCAT CAGTACCTTT
421 CACCTGTCCA TCCCCAATTT CAACCAGTAC GAGGCAATGT CATGCGACTT CAACGGGGGC
481 AAGATCAGTG TCCAGTACAA CCTGAGCCAC TCCTACGCCG GCGACGCAGC CAACCACTGC
541 GGAACTGTCG CCAATGGCGT GCTGCAGACA TTCATGAGGA TGGCATGGGG GGGATCTTAC
601 ATCGCACTGG ATAGCGGCAG GGGCAATTGG GATTGCATCA TGACTTCCTA TCAGTATCTG
661 ATTATCCAGA ATACTACATG GGAGGATCAT TGCCAGTTCA GTCGGCCCAG CCCTATTGGA
721 TATCTGGGGC TGCTGTCACA GAGAACACGG GATATCTATA TTTCAAGACG CCTGCTGGGC
781 ACATTCACTT GGACACTGTC AGACAGTGAG GGCAAGGATA CTCCAGGGGG CTACTGCCTG
841 ACACGATGGA TGCTGATCGA AGCAGAGCTG AAATGCTTCG GCAATACCGC AGTGGCCAAG
901 TGCAACGAGA AACACGACGA GGAGTTCTGC GACATGCTGA GGCTGTTCGA CTTCAACAAA
961 CAGGCTATCC AGAGACTGAA GGCAGAAGCC CAGATGTCAA TCCAGCTGAT CAACAAGGCA
1021 GTGAACGCCC TGATCAACGA CCAGCTGATC ATGAAGAACC ACCTGAGAGA CATTATGGGC
1081 ATCCCCTACT GTAATTACAG CAAGTATTGG TACCTGAACC ACACTACAAC CGGGAGAACA
1141 TCCCTGCCCA AGTGCTGGCT GGTCAGCAAT GGGAGTTATC TGAATGAAAC CCATTTCAGC
1201 GACGATATCG AACAGCAGGC TGACAACATG ATCACAGAGA TGCTGCAGAA AGAGTACATG
1261 GAAAGACAGG GCAAGACACC ACTGGGACTG GTCGATCTGT TCGTCTTCTC CACTAGCTTC
1321 TATCTGATTT CCATCTTCCT GCACCTGGTG AAGATCCCCA CTCATAGGCA CATTGTCGGC
1381 AAGAGTTGCC CTAAACCCCA TAGGCTGAAT CACATGGGGA TTTGTAGTTG CGGCCTGTAT
1441 AAGCAGCCTG GCGTGCCTGT GAAATGGAAG AGATGA

SEQ ID NO: 3

SEQ ID No: 3 corresponds to a recombinant NP protein of the Lassa Virus strain Josiah encoded by the codon-optimised sequence of SEQ ID No: 4.

MSASKEIKSFLWTQSLRRELSGYCSNIKLQVVKDAQALLHGLDFSEVSNVQRL
MRKERRDDNDLKRLRDLNQAVNNLVELKSTQQKSILRVGTLTSDDLLILAADLE
KLKSKVIRTERPLSAGVYMGNLSSQQLDQRRALLNMIGMSGGNQGARAGRD
GVVRVWDVKNAELLNNQFGTMPSLTLACLTKQGQVDLNDAVQALTDLGLIYT
AKYPNTSDLDRLTQSHPILNMIDTKKSSLNISGYNFSLGAAVKAGACMLDGGN
MLETIKVSPQTMDGILKSILKVKKALGMFISDTPGERNPYENILYKICLSGDGWP
YIASRTSITGRAWENTVVDLESDGKPQKADSNNSSKSLQSAGFTAGLTYSQL
MTLKDAMLQLDPNAKTWMDIEGRPEDPVEIALYQPSSGCYIHFFREPTDLKQF
KQDAKYSHGIDVTDLFATQPGLTSAVIDALPRNMVITCQGSDDIRKLLESQGRK
DIKLIDIALSKTDSRKYENAVWDQYKDLCHMHTGVVVEKKKRGGKEEITPHCA
LMDCIMFDAAVSGGLNTSVLRAVLPRDMVFRTSTPRVVL*

SEQ ID NO: 4

SEQ ID No: 4 corresponds to a codon-optimized nucleotide sequence encoding the NP protein of SEQ ID No.3.

1 ATGAGTGCCA GCAAAGAAAT CAAGAGCTTC CTGTGGACCC AGAGTCTGCG GAGGGAACTG
61 AGCGGATACT GTAGCAACAT CAAACTGCAG GTGGTCAAGG ACGCTCAGGC ACTGCTGCAT
121 GGGCTGGACT TCTCCGAGGT GTCTAATGTG CAGCGGCTGA TGCGGAAAGA ACGGAGGGAC
181 GATAATGACC TGAAGCGACT GCGCGACCTG AACCAGGCAG TGAACAATCT GGTCGAGCTG
241 AAGAGCACCC AGCAGAAATC AATCCTGCGG GTCGGGACAC TGACATCTGA CGACCTGCTG
301 ATCCTGGCTG CAGACCTGGA GAAGCTGAAA TCGAAAGTGA TCCGCACCGA AAGGCCACTG
361 TCCGCCGGGG TCTACATGGG CAATCTGTCT TCCCAGCAGC TGGACCAGAG GCGGGCTCTG
421 CTGAACATGA TTGGGATGTC CGGAGGAAAT CAGGGAGCTA GAGCCGGGAG GGACGGAGTC
481 GTGCGGGTCT GGGACGTGAA GAATGCCGAA CTGCTGAACA ACCAGTTCGG GACCATGCCA
541 AGTCTGACAC TGGCATGCCT GACTAAACAG GGCCAGGTGG ATCTGAATGA TGCAGTCCAG
601 GCTCTGACCG ACCTGGGCCT GATCTACACC GCCAAGTACC CCAATACTAG CGACCTGGAT
661 AGACTGACCC AGAGCCACCC CATCCTGAAC ATGATCGACA CTAAGAAGTC CTCACTGAAC
721 ATCAGTGGCT ATAATTTCTC CCTGGGGGCA GCAGTCAAGG CTGGCGCATG CATGCTGGAC
781 GGCGGGAATA TGCTGGAAAC CATCAAAGTG TCTCCCCAGA CCATGGATGG CATCCTGAAA
841 TCTATTCTGA AAGTCAAGAA GGCCCTGGGA ATGTTTATTT CAGACACCCC CGGCGAGAGG
901 AATCCATATG AGAACATTCT GTATAAGATT TGCCTGAGTG GCGACGGGTG GCCATACATT
961 GCAAGCCGGA CATCAATTAC CGGAAGAGCT TGGGAGAATA CAGTCGTGGA CCTGGAAAGC
1021 GACGGCAAGC CCCAGAAGGC CGACTCAAAC AACTCCTCAA AGAGTCTGCA GTCAGCTGGC
1081 TTCACAGCAG GGCTGACTTA CTCCCAGCTG ATGACACTGA AGGACGCAAT GCTGCAGCTG
1141 GACCCAAACG CTAAGACATG GATGGACATC GAGGGACGGC CAGAAGATCC AGTGGAAATC
1201 GCACTGTATC AGCCATCATC CGGATGCTAT ATCCATTTCT TCCGGGAACC AACTGATCTG
1261 AAGCAGTTCA AGCAGGATGC AAAGTACTCC CACGGAATCG ATGTCACCGA TCTGTTCGCA
1321 ACCCAGCCAG GACTGACATC AGCCGTCATC GATGCCCTGC CTAGGAACAT GGTCATTACT

1381 TGCCAGGGCT CCGACGATAT TAGGAAGCTG CTGGAGAGCC AGGGACGGAA GGATATCAAA
1441 CTGATCGATA TTGCCCTGTC TAAGACTGAT AGCCGGAAAT ATGAGAATGC AGTCTGGGAT
1501 CAGTACAAGG ACCTGTGCCA TATGCATACC GGAGTGGTCG TCGAGAAGAA GAAGAGGGGC
1561 GGAAAGGAAG AGATCACACC CCACTGTGCC CTGATGGATT GCATCATGTT CGACGCAGCC
1621 GTGTCCGGGG GCCTGAACAC CTCAGTCCTG AGGGCTGTCC TGCCAAGAGA TATGGTGTTT
1681 AGAACTTCAA CCCCAAGAGT CGTCCTGTAA

SEQ ID NO: 5

SEQ ID No: 5 corresponds to a recombinant mutated NP protein of the Lassa Virus strain Josiah encoded by the codon-optimised sequence of SEQ ID No: 6, and wherein the exonuclease activity of the NP protein has been knocked down. Amino acid 388 and amino acid 391 have been mutated (M388D and E391G).

MSASKEIKSFLWTQSLRRELSGYCSNIKLQVVKDAQALLHGLDFSEVSNVQRL
MRKERRDDNDLKRLRDLNQAVNNLVELKSTQQKSILRVGTLTSDDLLILAADLE
KLKSKVIRTERPLSAGVYMGNLSSQQLDQRRALLNMIGMSGGNQGARAGRD
GVVRVWDVKNAELLNNQFGTMPSLTLACLTKQGQVDLNDAVQALTDLGLIYT
AKYPNTSDLDRLTQSHPILNMIDTKKSSLNISGYNFSLGAAVKAGACMLDGGN
MLETIKVSPQTMDGILKSILKVKKALGMFISDTPGERNPYENILYKICLSGDGWP
YIASRTSITGRAWENTVVDLESDGKPQKADSNNSSKSLQSAGFTAGLTYSQL
MTLKDAMLQLDPNAKTWM**A**IE**A**RPEDPVEIALYQPSSGCYIHFFREPTDLKQF
KQDAKYSHGIDVTDLFATQPGLTSAVIDALPRNMVITCQGSDDIRKLLESQGRK
DIKLIDIALSKTDSRKYENAVWDQYKDLCHMHTGVVVEKKKRGGKEEITPHCA
LMDCIMFDAAVSGGLNTSVLRAVLPRDMVFRTSTPRVVL*

SEQ ID NO: 6

SEQ ID No: 6 corresponds to a codon-optimized nucleotide sequence encoding the mutated NP protein of SEQ ID No.5. Nucleotides 11661 1175 and 1176 has been mutated (C1166A, C1175G and C1176A).

1 ATGAGTGCCA GCAAAGAAAT CAAGAGCTTC CTGTGGACCC AGAGTCTGCG GAGGGAACTG
61 AGCGGATACT GTAGCAACAT CAAACTGCAG GTGGTCAAGG ACGCTCAGGC ACTGCTGCAT
121 GGGCTGGACT TCTCCGAGGT GTCTAATGTG CAGCGGCTGA TGCGGAAAGA ACGGAGGGAC
181 GATAATGACC TGAAGCGACT GCGCGACCTG AACCAGGCAG TGAACAATCT GGTCGAGCTG
241 AAGAGCACCC AGCAGAAATC AATCCTGCGG GTCGGGACAC TGACATCTGA CGACCTGCTG
301 ATCCTGGCTG CAGACCTGGA GAAGCTGAAA TCGAAAGTGA TCCGCACCGA AAGGCCACTG
361 TCCGCCGGGG TCTACATGGG CAATCTGTCT TCCCAGCAGC TGGACCAGAG GCGGGCTCTG

421 CTGAACATGA TTGGGATGTC CGGAGGAAAT CAGGGAGCTA GAGCCGGGAG GGACGGAGTC
481 GTGCGGGTCT GGGACGTGAA GAATGCCGAA CTGCTGAACA ACCAGTTCGG GACCATGCCA
541 AGTCTGACAC TGGCATGCCT GACTAAACAG GGCCAGGTGG ATCTGAATGA TGCAGTCCAG
601 GCTCTGACCG ACCTGGGCCT GATCTACACC GCCAAGTACC CCAATACTAG CGACCTGGAT
661 AGACTGACCC AGAGCCACCC CATCCTGAAC ATGATCGACA CTAAGAAGTC CTCACTGAAC
721 ATCAGTGGCT ATAATTTCTC CCTGGGGGCA GCAGTCAAGG CTGGCGCATG CATGCTGGAC
781 GGCGGGAATA TGCTGGAAAC CATCAAAGTG TCTCCCCAGA CCATGGATGG CATCCTGAAA
841 TCTATTCTGA AAGTCAAGAA GGCCCTGGGA ATGTTTATTT CAGACACCCC CGGCGAGAGG
901 AATCCATATG AGAACATTCT GTATAAGATT TGCCTGAGTG GCGACGGGTG GCCATACATT
961 GCAAGCCGGA CATCAATTAC CGGAAGAGCT TGGGAGAATA CAGTCGTGGA CCTGGAAAGC
1021 GACGGCAAGC CCCAGAAGGC CGACTCAAAC AACTCCTCAA AGAGTCTGCA GTCAGCTGGC
1081 TTCACAGCAG GGCTGACTTA CTCCCAGCTG ATGACACTGA AGGACGCAAT GCTGCAGCTG
1141 GACCCAAACG CTAAGACATG GATGG**C**CATC GAGG**CC**CGGC CAGAAGATCC AGTGGAAATC
1201 GCACTGTATC AGCCATCATC CGGATGCTAT ATCCATTTCT TCCGGGAACC AACTGATCTG
1261 AAGCAGTTCA AGCAGGATGC AAAGTACTCC CACGGAATCG ATGTCACCGA TCTGTTCGCA
1321 ACCCAGCCAG GACTGACATC AGCCGTCATC GATGCCCTGC CTAGGAACAT GGTCATTACT
1381 TGCCAGGGCT CCGACGATAT TAGGAAGCTG CTGGAGAGCC AGGGACGGAA GGATATCAAA
1441 CTGATCGATA TTGCCCTGTC TAAGACTGAT AGCCGGAAAT ATGAGAATGC AGTCTGGGAT
1501 CAGTACAAGG ACCTGTGCCA TATGCATACC GGAGTGGTCG TCGAGAAGAA GAAGAGGGGC
1561 GGAAAGGAAG AGATCACACC CCACTGTGCC CTGATGGATT GCATCATGTT CGACGCAGCC
1621 GTGTCCGGGG GCCTGAACAC CTCAGTCCTG AGGGCTGTCC TGCCAAGAGA TATGGTGTTT
1681 AGAACTTCAA CCCCAAGAGT CGTCCTGTAA

SEQ ID NO: 7

SEQ ID No: 7 corresponds to a recombinant Z protein of the Lassa Virus strain Josiah encoded by the codon-optimised sequence of SEQ ID No: 8.

MGNKQAKAPESKDSPRASLIPDATHLGPQFCKSCWFENKGLVECNNHYLCLN
CLTLLLSVSNRCPICKMPLPTKLRPSAAPTAPPTGAADSIRPPPYSP*

SEQ ID NO: 8

SEQ ID No: 8 corresponds to a codon-optimized nucleotide sequence encoding the Z protein of SEQ ID No.7.

1 ATGGGCAATA AGCAGGCAAA GGCACCCGAA AGCAAGGATT CACCTAGAGC ATCACTGATT
61 CCCGACGCAA CTCATCTGGG GCCACAGTTC TGCAAATCCT GTTGGTTCGA GAACAAAGGC
121 CTGGTGGAGT GCAATAACCA CTACCTGTGC CTGAACTGTC TGACACTGCT GCTGAGTGTG
181 AGCAACAGAT GCCCAATCTG CAAGATGCCT CTGCCAACAA AGCTGAGGCC TTCTGCTGCA
241 CCCACCGCAC CACCAACTGG AGCCGCAGAC AGCATTAGAC CCCCCCCATA CTCACCATAA

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CLAIMS

1. A nucleic acid construct which comprises:

(1) a cDNA molecule encoding a full length antigenomic (+) RNA strand of a measles virus (MeV);

(2) a first heterologous polynucleotide encoding at least a glycoprotein precursor (GPC) of a Lasa Virus (LASV); and a mutated nucleoprotein (mNP) of LASV knocked down for its exonuclease activity, or

(2') a first heterologous polynucleotide encoding at least a glycoprotein precursor (GPC) of LASV, and a second heterologous polynucleotide encoding at least a Zinc-binding protein (Z protein) of LASV,

wherein the first heterologous polynucleotide is operatively cloned within an additional transcription unit (ATU) inserted within the cDNA of the antigenomic (+) RNA,

wherein the second heterologous polynucleotide when present is operatively cloned within another ATU at a location distinct from the location of the first cloned heterologous polynucleotide.

2. The nucleic acid construct according to claim 1

wherein the first heterologous polynucleotide is operatively cloned within an ATU localized between the P gene and the M gene of the MeV, in particular the ATU2 inserted between the P gene and the M gene of the MeV.

3. The nucleic acid construct according to claim 2 wherein the first heterologous polynucleotide is operatively cloned with the ATU2 and the second heterologous polynucleotide is operatively cloned within the ATU1.

4. The nucleic acid construct according to any one of claims 1 to 3, wherein the heterologous polynucleotide(s) encoding the GPC protein, the mNP

and/or the Z protein, is(are) from the LASV strain *Josiah*, or is(are) derived from the LASV strain *Josiah*, in particular the heterologous polynucleotide(s) is(are) issued or derived from the sequence of Genbank J04324.1 and/or U73034.2.

5. The nucleic acid construct according to any one of claims 1 to 4, wherein the first and/or, when applicable, the second heterologous polynucleotide(s), encode(s) the mNP protein, wherein the amino acid sequence of the encoded mNP protein is mutated on amino acid residue 389 and/or 392, preferentially by substitution on amino acid residues 389 and 392, of SEQ ID No: 3, in particular the encoded mNP protein is the sequence of SEQ ID No: 5, in particular the heterologous polynucleotide encoding the mNP protein comprises SEQ ID No: 6.

6. The nucleic acid construct according to any one of claims 1 to 5, wherein the heterologous polynucleotide(s) encoding the GPC protein, the mNP protein and/or the Z protein, has (have) codon-optimized open reading frame(s) (ORF), in particular, the heterologous polynucleotide(s) comprise(s) at least one of the following sequence:
   - SEQ ID No: 2 which encodes the GPC protein; and/or
   - SEQ ID No: 6 which encodes the mNP protein; and/or
   - SEQ ID No: 8 which encodes the Z protein.

7. The nucleic acid construct according to any one of claims 1 to 6, wherein the heterologous polynucleotide(s) encode(s):
   - the GPC protein of SEQ ID No: 1; and/or
   - the mNP protein of SEQ ID No: 5; and/or
   - the Z protein of SEQ ID No: 7.

8. The nucleic acid construct according to any one of claims 1 to 7 comprising from 5’ to 3’ end the following polynucleotides:
   (a) a polynucleotide encoding the N protein of the MeV;

(b) a polynucleotide encoding the P protein of the MeV;
(c) the first heterologous polynucleotide encoding at least two polypeptides, which are the GPC protein and the mNP protein, wherein the first polynucleotide is in particular operatively cloned within an ATU, in particular ATU2;
(d) a polynucleotide encoding the M protein of the MeV;
(e) a polynucleotide encoding the F protein of the MeV;
(f) a polynucleotide encoding the H protein of the MeV;
(g) a polynucleotide encoding the L protein of the MeV;
and wherein said polynucleotides are operatively linked within the nucleic acid construct and under the control of a viral replication and transcriptional regulatory elements such as MeV leader and trailer sequence(s).

9. The nucleic acid construct according to any one of claims 2 to 7 comprising from 5' to 3' end the following polynucleotides:
(a) the second heterologous polynucleotide encoding at the Z protein, wherein the second polynucleotide is operatively cloned within an ATU localized upstream the N gene of the MeV, in particular within the ATU1;
(b) a polynucleotide encoding the N protein of the MeV;
(c) a polynucleotide encoding the P protein of the MeV;
(d) the first heterologous polynucleotide encoding at least the GPC protein, wherein the first polynucleotide is in particular operatively cloned within an ATU, in particular ATU2;
(e) a polynucleotide encoding the M protein of the MeV;
(f) a polynucleotide encoding the F protein of the MeV;
(g) a polynucleotide encoding the H protein of the MeV;
(h) a polynucleotide encoding the L protein of the MeV,
wherein said polynucleotides are operatively linked within the nucleic acid construct and under the control of a viral replication and transcriptional regulatory elements such as MeV leader and trailer sequence(s).

10. The nucleic acid construct according to any one of claims 1 to 8, wherein the first heterologous polynucleotide comprises from 5' to 3' end:
(a) a nucleic acid encoding the mNP protein of the LASV, in particular whose nucleic acid is SEQ ID No:; and
(b) a nucleic acid encoding the GPC protein of the LASV, in particular whose nucleic acid is SEQ ID No: 2;
and wherein the first heterologous polynucleotide is operatively cloned between the P gene and the M gene of the MeV, in particular within an ATU, in particular ATU2.

11. The nucleic acid construct according to any one of claims 1 to 9, wherein the first heterologous polynucleotide comprises from 5' to 3' end:
(a) the nucleic acid of SEQ ID No: 6 encoding the mNP protein; and
(b) the nucleic acid of SEQ ID No: 2 encoding the GPC protein;
and wherein the first heterologous polynucleotide sequence is operatively cloned between the gene P and the gene M of the MeV, in particular within an ATU, in particular ATU2.

12. The nucleic acid construct according to any one of claims 2 to 11, wherein the second heterologous polynucleotide encodes the Z protein of the LASV, and wherein the first heterologous polynucleotide encodes the GPC protein of the LASV, in particular wherein the sequence of the second heterologous polynucleotide comprises the sequence of SEQ ID No: 8 and the sequence of the first heterologous polynucleotide comprises the sequence of SEQ ID No: 2.

13. The nucleic acid construct according to any one of claims 1 to 12, wherein the measles virus is an attenuated virus strain selected from the group consisting of the Schwarz strain, the Zagreb strain, the AIK-C strain, the Moraten strain, the Philips strain, the Beckenham 4A strain, the Beckenham 16 strain, the Edmonston seed A strain, the Edmonston

seed B strain, the CAM-70 strain, the TD 97 strain, the Leningrad-16 strain, the Shanghai 191 strain and the Belgrade strain, in particular the Schwarz strain.

14. The nucleic acid construct according to any one of claims 1 to 13, or a transfer plasmid vector comprising the nucleic acid construct according to any one of claims 1 to 13, whose recombinant cDNA sequence is selected from the group consisting of:
    - SEQ ID No: 11 (construct MeV-mNP-GPC);
    - SED ID No: 12 (construct Z-MeV-GPC); and
    - SEQ ID No: 14 (construct Z-MeV-mNP-GPC).

15. A recombinant measles virus comprising in its genome or whose genome consists of a nucleic acid construct according to any one of claims 1 to 14, or a transfer plasmid vector according to claim 14, or whose genome consists of the transfer plasmid vector of claim 14.

16. The recombinant measles virus according to claim 15 expressing at least two polypeptides, one being the GPC protein, the other being selected from the group consisting of the mNP protein or the Z protein of the LASV, or in particular expressing the GPC protein and the Z protein of the LASV, or in particular expressing the GPC protein and the mNP protein of the LASV.

17. The recombinant measles virus according to claim 15 or 16 furthermore expressing at least one of the N protein, the P protein, the M protein, the F protein, the H protein and the L protein of the MeV.

18. The recombinant measles virus according to any one of claims 15 to 17 , which elicits a cellular and/or humoral and cellular response, in particular after a single immunization, against the GPC protein, the mNP protein and/or the Z protein of LASV, in particular against the N protein, the P

protein, the M protein, the F protein, the H protein and/or the L protein of MeV, in particular a T cell response, in particular a CD4+ and CD8+ T cell response.

19. A host cell transfected with the nucleic acid construct according to any one of claims 1 to 14 or with the transfer plasmid vector according to claim 14, or infected with the recombinant measles virus according to any one of claims 15 to 18, in particular a mammalian cell, a VERO NK cells, CEF cells, human embryonic kidney cell line 293 or MRC5 cells.

20. An immunogenic composition, especially a virus vaccine composition, comprising the recombinant measles virus according to any one of claims 15 to 18, and a pharmaceutically acceptable vehicle.

21. The composition according to claim 20 for use in the elicitation of a protective, and preferentially prophylactic, immune response against the Lassa virus and/or Measles virus by the elicitation of antibodies directed against LASV protein(s) and/or Measles virus protein(s), and/or a cellular and/or humoral and cellular response against the Lassa virus and/or the Measles virus, in a host in need thereof, in particular a human host, in particular a child.

22. A process for rescuing recombinant Lassa virus like particles (VLPs) and/or recombinant measles virus expressing at least the GPC protein of LASV, and one of polypeptide of LASV selected from the group consisting the mNP protein and the Z protein, comprising:
    (a) transfecting cells, in particular helper cells, in particular HEK293 helper cells, stably expressing T7 RNA polymerase and measles virus N and P proteins with the nucleic acid construct according to any one of claims 1 to 14 or with the transfer plasmid vector according to claim 14;

(b) maintaining the transfected cells in conditions suitable for the production of recombinant measles virus and/or LASV VLPs;

(c) infecting cells enabling propagation of the recombinant measles virus and/or the LASV VLPs by co-cultivating them with the transfected cells of step (b);

(d) harvesting the recombinant measles virus expressing at least the GPC protein and the mNP protein and/or the NP protein and/or the Z protein, and/or the LASV VLPs expressing at least the Z protein and the GPC protein, and optionally the NP protein and/or the mNP protein of LASV.

23. Use of a measles virus according to any one of claims 15 to 18 in the manufacture of a medicament for preventing an infection by a Lassa virus.

24. Use of a measles virus according to any one of claims 15 to 18 in the manufacture of a medicament for treating an infection by a Lassa virus.