

Rhinovirus Vaccine

The present invention relates to immunogenic compositions, and in particular, to immunogenic compositions for preventing, treating or ameliorating human rhinovirus (RV) infections. The invention is especially concerned with RV VPo peptides (or
5 proteins) and polynucleotides encoding such peptides, and their use in immunogenic compositions for eliciting an immune response and preventing rhinovirus infections.

Rhinoviruses (RVs) are picornaviruses, which are small single stranded RNA viruses with a genome size of approximately 6.8-7.2 Kb [1]. RVs are the major cause of
10 common colds, of virus-induced wheezing illnesses in early childhood and of acute attacks (exacerbations) of lung diseases such as asthma, bronchiectasis, chronic obstructive pulmonary disease (COPD), cystic fibrosis and chronic fibrosing lung diseases [2,3]. RVs cause around 75% to 90% of such illnesses.

15 There are currently no effective anti-RV treatments and no anti-RV vaccines. A major obstacle to the development of a RV vaccine has been the large number of genetically and antigenically distinct circulating strains. A recent study estimated that approximately 180 genetically distinct strains of RV are in circulation [4]. These strains are classified based on their genome identity into three species: RV-A, RV-B and RV-C
20 [1,5]. RV-A and RV-C are the largest species numerically and are most important in terms of clinical illness, with RV-A and RV-Cs responsible for most exacerbations of asthma and COPD and childhood wheezing illnesses resulting in hospitalisation. However, RV-Bs are also associated with an increased risk of wheezing illnesses [7].

25 There is, therefore, a need to provide an immunogenic composition that is capable of protecting individuals against many, if not all, RV strains.

The inventors, using in depth bioinformatics analysis, have identified RV VPo proteins from single representative strains that, when used as vaccine immunogens, surprisingly
30 evoke cellular immunity against all the other members of that RV species.

Therefore, according to a first aspect of the invention, there is provided an immunogenic composition comprising at least one isolated human rhinovirus (RV) VPo peptide, or an isolated polynucleotide encoding the peptide, wherein the peptide is
35 selected from a group consisting of: an RV-C VPo peptide; an RV-B VPo peptide; RV-A28 VPo; and RV-A89 VPo, or a variant or fragment thereof.

- 2 -

As shown in the examples, the inventors surprisingly discovered that the novel rhinovirus immunogens are able to elicit broad cellular immune responses that cross-react with other RV strains from the same species. For example, as shown in Figures 4, 8, and 9 splenocytes from RV-Bo6 VPo immunised mice cross react with VPo peptides from other RV-B species members, and splenocytes from either RV-C19 VPo or RV-C24 VPo immunised mice cross react with VPo peptides from other RV-C species members, respectively. Additionally, the inventors have demonstrated that immunisation with mRNA encoding the RV VPo peptides, elicits a broad cellular immune response. For example, as shown in Figure 15, the inventors demonstrated that immunisation of mice with RV-C24 VPo mRNA evokes cellular immunity that is cross-reactive with other members of C species of RV. Advantageously, therefore, the immunogenic composition according to the invention overcomes the issue of antigenic heterogeneity across RV strains, which has significantly hampered RV vaccine development to date.

In a preferred embodiment, the immunogenic composition comprises an RV-C VPo peptide.

In another preferred embodiment, the immunogenic composition comprises an RV-B VPo peptide.

In another preferred embodiment, the immunogenic composition comprises RV-A28 VPo and/or RV-A89 VPo.

Alternatively, in another embodiment, the immunogenic composition comprises at least two isolated human rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the peptides, wherein the peptides are selected from a group consisting of: an RV-C VPo peptide; an RV-B VPo peptide; RV-A28 VPo; and RV-A89 VPo.

In another preferred embodiment, the immunogenic composition comprises at least three isolated human rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the peptides, wherein the peptides are selected from a group consisting of: an RV-C VPo peptide; an RV-B VPo peptide; RV-A28 VPo; and RV-A89 VPo.

Preferably, the RV-C VPo peptide is selected from a group consisting of: RV-Co7 VPo; RV-C19 VPo; RV-C24 VPo; and RV-Co1 VPo, or a variant or fragment thereof.

- 3 -

Preferably, the RV-B VPo peptide is RV-Bo6 VPo.

Accordingly, in one embodiment, the immunogenic composition preferably comprises
5 at least two isolated human rhinovirus (RV) VPo peptides, or isolated polynucleotides
encoding the peptides, wherein the peptides are selected from a group consisting of:
RV-A28; RV-A89; RV-Bo6 VPo; RV-Co7 VPo; RV-C19 VPo; RV-C24 VPo; and RV-Co1
VPo, or a variant or fragment thereof.

10 Alternatively, the immunogenic composition preferably comprises at least three
isolated human rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the
peptides, wherein the peptides are selected from a group consisting of: RV-A28; RV-
A89; RV-Bo6 VPo; RV-Co7 VPo; RV-C19 VPo; RV-C24 VPo; and RV-Co1 VPo, or a
variant or fragment thereof.

15 Alternatively, the immunogenic composition preferably comprises at least four isolated
human rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the
peptides, wherein the peptides are selected from a group consisting of: RV-A28; RV-
A89; RV-Bo6 VPo; RV-Co7 VPo; RV-C19 VPo; RV-C24 VPo; and RV-Co1 VPo, or a
20 variant or fragment thereof.

Alternatively, the immunogenic composition comprises at least five isolated human
rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the peptides,
wherein the peptides are selected from a group consisting of: RV-A28; RV-A89; RV-
25 Bo6 VPo; RV-Co7 VPo; RV-C19 VPo; RV-C24 VPo; and RV-Co1 VPo, or a variant or
fragment thereof.

Alternatively, the immunogenic composition comprises at least six isolated human
rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the peptides,
30 wherein the peptides are selected from a group consisting of: RV-A28; RV-A89; RV-
Bo6 VPo; RV-Co7 VPo; RV-C19 VPo; RV-C24 VPo; and RV-Co1 VPo, or a variant or
fragment thereof.

Alternatively, the immunogenic composition comprises at least seven isolated human
35 rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the peptides,
wherein the peptides are selected from a group consisting of: RV-A28; RV-A89; RV-

- 4 -

Bo6 VPo; RV-Co7 VPo; RV-C19 VPo; RV-C24 VPo; and RV-Co1 VPo, or a variant or fragment thereof.

In one embodiment, the immunogenic composition may further comprise an isolated
5 RV-A16 VPo peptide and/or an isolated RV-A29 VPo peptide, or an isolated polynucleotide encoding the peptide, or a variant or fragment thereof.

In a preferred embodiment, the immunogenic composition according to the first aspect
10 comprises three isolated human rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the peptides, wherein the peptides are selected from a group consisting of: RV-A16 VPo; RV-Bo6 VPoRV; and RV-C24 VPo, or a variant or fragment thereof.

It is well-known to the skilled person that there are three different species of
15 rhinoviruses: rhinovirus A (RV-A), which is also called type A rhinovirus, rhinovirus B (RV-B), which is also called type B rhinovirus, and rhinovirus C (RV-C), which is also called type C rhinovirus. RVs are further classified into strains according to their nucleotide sequence homologies. As used herein, the term "strain" refers to a subdivision within a species of rhinoviruses and relies on the VP1 gene sequence of the
20 rhinovirus. For example, the terms RV-A16, RV-A28, RV-A29, RV-A89, RV-Bo6, RV-Co7, RV-C19, RV-C24, and RV-Co1, refer to the RV strain. RVs have been classified according to several other parameters, including receptor specificity and antiviral susceptibility.

25 RVs have a 25 nm capsid of icosahedral symmetry, made up of 60 copies of each of four virus-coded proteins (VP1, VP2, VP3 and VP4) and enclosing a single-stranded RNA genome of approximately 7,500 nucleotides. The RNA is of positive polarity, is polyadenylated at its 3' terminus and is covalently bound at its 5' terminal end to a small protein, VPg. The primary translational product of this RNA is a single, "large"
30 polyprotein, divided into three smaller polyproteins called, P1, P2 and P3, which are subsequently processed by proteolytic cleavage to yield the mature virus proteins. The P1 polyprotein is composed of four peptides (1A or VP4, 1B or VP2, 1C or VP3, and 1D or VP1), the P2 polyprotein is composed of three peptides (2A, 2B and 2C) and the P3 polyprotein is composed of four peptides (3A, 3B, 3C and 3D). 2A and 3C are viral
35 proteases, while 3D corresponds to the viral RNA polymerase. The P1 polyprotein is the precursor that gives rise to the four structural proteins of the nucleocapsid. The P1

- 5 -

polyprotein is first cleaved to produce the VPo polyprotein, which contains the amino acid sequence of VP4 and VP2 peptides, the VP3 peptide and the VP1 peptide. The VPo polyprotein is then cleaved into the VP4 peptide and the VP2 peptide once the virus has assembled.

5

It will be well known to the skilled person that the term "VPo polyprotein", "VPo peptide" or "peptide 1AB" refers to the protein precursor derived from the RV P1 polyprotein and which consists of the amino acid sequence of VP4 and VP2 peptides. VPo polyprotein is typically about 330 amino acids long. The amino acid sequence of
10 the VPo polyprotein slightly varies according to the RV strain or species.

Accordingly, a fragment of the RV VPo peptide may comprise or consist of the corresponding VP2 peptide and/or VP4 peptide. Thus, a fragment of the RV VPo may have at least 1, 2, 3, 4, 5, 10, 20, 25, 30, 45, 50, 60, 70, 80, 90 or 100 amino acids fewer
15 than the full length RV VPo described herein.

In one embodiment, the peptide, variant or fragment thereof, comprises or consists of at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 66 amino acids, at
20 least 67 amino acids, at least 68 amino acids, or at least 69 amino acids.

In another embodiment, the peptide, variant or fragment thereof, comprises or consists of at least 70 amino acids, at least 80 amino acids, at least 90 amino acids, at least 100 amino acids, at least 110 amino acids, at least 120 amino acids, at least 130 amino acids,
25 at least 140 amino acids, at least 150 amino acids, at least 160 amino acids, at least 170 amino acids, at least 180 amino acids, at least 190 amino acids, at least 200 amino acids, at least 210 amino acids, at least 220 amino acids, at least 230 amino acids, at least 240 amino acids, at least 250 amino acids, at least 255 amino acids, at least 260 amino acids, at least 261 amino acids, at least 262 amino acids, at least 263 amino
30 acids, at least 264 amino acids, at least 265 amino acids, at least 266 amino acids, or at least 267 amino acids.

In another embodiment, the peptide, variant or fragment thereof, comprises or consists of at least 280 amino acids, at least 290 amino acids, at least 300 amino acids, at least
35 310 amino acids, at least 320 amino acids, or at least 330 amino acids.

- 6 -

As used herein, the term “isolated” means removed from the natural environment, i.e. from rhinoviruses or cells infected by a rhinovirus. Usually, it refers to a peptide or a nucleic acid substantially free of cellular material, bacterial material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical
5 precursors, or other chemicals when chemically synthesized.

In one embodiment, at least two or more of the RV VPo peptides may form a fusion peptide or protein. It is well-known to the skilled person that a fusion peptide/protein is a peptide comprising all or part of the amino acid sequence of at least two or more
10 individual peptide units linked together via a covalent linkage, *e.g.* via peptide (amide) bonds. The two or more peptides may be joined directly to each other or they may be joined using a linker sequence. In another embodiment, at least three, four or five or more of the RV VPo peptides may form a fusion peptide. Alternatively, a plurality of RV VPo peptides may be kept as separate peptides, but used simultaneously, i.e. not fused
15 together.

The present invention also relates to an immunogenic composition comprising an isolated polynucleotide(s) encoding the isolated human rhinovirus (RV) VPo peptide(s).
20

In a preferred embodiment, the polynucleotide comprises a nucleic acid sequence encoding the peptide. Preferably, the nucleic acid sequence is placed under the control of the elements necessary for its expression in a mammalian cell, in particular in human cells. The nucleic acid sequence may be incorporated in a plasmid, which can be
25 further formulated in a delivery vehicle such as liposomes to facilitate its introduction into the host cell.

As used herein, the term “nucleic acid” includes DNA and RNA, such as messenger RNA (mRNA) or self-amplifying RNA (saRNA), and can be either double-stranded or
30 single-stranded. Most preferably, the nucleic acid is mRNA.

As used herein, the expression “elements necessary for expression in a mammalian cell” is understood to mean all the elements which allow the transcription of a DNA or DNA fragment into mRNA or saRNA and the translation of the latter into protein, inside a
35 mammalian cell, such as a human cell. Typically, the elements necessary for the expression of a nucleic acid in a mammalian cell include a promoter that is functional

- 7 -

in the selected mammalian cell and can be constitutive or inducible; a ribosome binding site; a start codon (ATG) if necessary; a region encoding a signal peptide (e.g., a lipidation signal peptide); a stop codon; and a 3' terminal region (translation and/or transcription terminator). Other transcription control elements, such as enhancers, operators, and repressors can be also operatively associated with the polynucleotide to direct transcription and/or translation into the cell. The signal peptide-encoding region is preferably adjacent to the nucleic acid included in the immunogenic composition of the invention and placed in proper reading frame. The signal peptide-encoding region can be homologous or heterologous to the DNA molecule encoding the mature peptide or fusion peptide of the invention and can be specific to the secretion apparatus of the host used for expression. The open reading frame constituted by the nucleic acid included in the immunogenic composition of the invention, solely or together with the signal peptide, is placed under the control of the promoter so that transcription and translation occur in the host system. Promoters, (and signal peptide encoding regions) are widely known and available to those skilled in the art.

The nucleic acid sequences may be codon optimized such that the transcription of the DNA encoding the peptides and/or the fusion peptides of the invention is enhanced and/or the translation of the mRNA encoding the peptides and/or the fusion peptides is prolonged.

The immunogenic composition may comprise a concatemer of the isolated polynucleotides encoding the RV VPo peptides. In a preferred embodiment, the polynucleotides encoding the RV VPo peptides are mRNA. Accordingly, in one embodiment, the immunogenic composition comprises an isolated polynucleotide encoding at least two or more of the RV VPo peptides. In another embodiment, the polynucleotide encodes at least three, four or five or more of the RV VPo peptides. Alternatively, a plurality of RV VPo peptides may be encoded by a plurality of separate polynucleotides, but used simultaneously, i.e. not fused together.

The inventors discovered that VPo peptides from RV-A and RV-B species, elicited a stronger immune response when compared with VPo peptides from RV-C groups. As such, the inventors believe that a concatemer in which the RV-C VPo peptides are encoded first may be beneficial, as this will allow more of the RV-C VPo peptides to be expressed. Accordingly, in one embodiment, the nucleic acid sequence encoding the

- 8 -

RV-C VPo peptide is disposed 5' of the nucleic acid sequence encoding the RV-B VPo peptide, the RV-A28 VPo peptide and/or the RV-A89 VPo peptide.

Alternatively, in another embodiment, the nucleic acid sequences encoding the RV-A
5 VPo peptide, the RV-B VPo peptide, and the RV-C VPo peptide are disposed in any order.

In one preferred embodiment, the immunogenic composition comprises an isolated
10 polynucleotide encoding the RV-C24 VPo peptide, an isolated polynucleotide encoding the RV-A16 VPo peptide, and an isolated polynucleotide encoding the RV-Bo6 VPo peptide. Preferably, the polynucleotides encoding the RV VPo peptides are separated by a 2A peptide-encoding sequence.

In one embodiment, the amino acid sequence of the RV-A16 VPo peptide is provided
15 herein as SEQ ID No: 1, as follows:

20 MGAQVSRQNVGTHSTQNMVSNGLNYFNINYPKDAASSGASRLDFSQDPSKFTDPVKDVLEKGIPTLQS
PSVEACGYSDRIIQITRGDSTITSQDVANAVVGYGWPHYLTQDATAIDKPTQPDTSNRFYTLDSKMW
NSTSKGWWWKLPDALKDMGIFGENMFYHFLGRSGYTVHVQCNASKFHQGTLLVVMIPHQLATVNGNVN
AGYKYTHPGEAGREVGTVQVENEKQPSDDNWLNFDTLLGNLLIFPHQFINLRSNNSATLIVPYVNAVPMDS
SMVRHNNWSLVII PVCQLQSNNISNIVPITVSI SPMCAEFSGARAKTVVQ

[SEQ ID No: 1]

Accordingly, preferably the RV-A16 VPo peptide comprises an amino acid sequence
25 substantially as set out in SEQ ID No: 1, or a variant or fragment thereof.

The VP4 peptide of RV-A16 constitutes amino acid residues 1-69 of SEQ ID No: 1 and
the VP2 peptide of RV-A16 constitutes amino acid residues 70-330 of SEQ ID No: 1.
Thus, in embodiments in which the immunogenic composition comprises a fragment of
30 RV-A16 VPo, the fragment may comprise or consist of the amino acids forming the VP2
and/or VP4 peptides.

In one embodiment, the RV-A16 VPo peptide is encoded by the nucleotide sequence
(DNA) of SEQ ID No: 2, as follows:

35 ATGGGTGCGCAGGTGAGCCGTCAGAACGTGGGCACCCATAGCACCCAGAACATGGTGAGCAACGGCAGCA
GCCTGAACATATTTCAACATCAACTATTTCAAAGATGCGGCGAGCAGCGGTGCGAGCCGTCTGGATTTAG
CCAGGATCCGAGCAAAATTTACCGATCCGGTGAAAGATGTGCTGGAAAAAGGCATTCGACCCCTGCAGAGC
40 CCGAGCGTGGAAGCGTGCGGCTATAGCGATCGTATTATTAGATTACCCGTGGCGATAGCACCATACCA
GCCAGGATGTGGCGAACGCGGTGGTGGGCTATGGTGTGTGGCCGCAATATCTGACCCCGCAGGATGCGAC

- 9 -

CGCGATTGATAAACCGACCCAGCCGGATACCAGCAGCAACCGTTTTTATACCTGGATAGCAAAATGTGG
AACAGCACCAGCAAAGTTGGTGGTGAAACTGCCGGATGCGCTGAAAGATATGGGCATTTTCGGCGAAA
ACATGTTCTATCATTTTCTGGGCCGTAGCGGCTATACCGTGCATGTGCAGTGCAACGCGAGCAAAATTTCA
TCAGGGCACCCTGCTGGTTGTGATGATTCCGGAACATCAGCTGGCCACCGTGAAACAAAGGCAACGTGAAC
5 GCGGGCTATAAATATACCCATCCGGGCGAAGCGGGTCGTGAAGTGGGCACCCAGGTGGAAACGAAAAAC
AGCCGAGCGATGATAACTGGCTGAACTTTGATGGCACCCCTGCTGGGCAACCTGCTGATTTTCCGCATCA
GTTTCATTAACTGCGTAGCAACAACAGCGCGACCCTGATTGTGCGGTATGTGAACGCGGTGCCGATGGAT
AGCATGGTGCGTCATAACAATTGGAGCCTGGTGATTATTCCGGTGTGCCAGCTGCAGAGCAACAACATTA
GCAACATTGTGCCGATTACCGTGAGCATTAGCCCGATGTGCGCGGAATTTAGCGGTGCGCGTGCGAAAAAC
10 CGTGGTGCAG

[SEQ ID No: 2]

Accordingly, preferably the RV-A16 VPo peptide is encoded by the nucleotide sequence
substantially as set out in SEQ ID No: 2, or a variant or fragment thereof. Alternatively,
15 the immunogenic composition comprises an isolated polynucleotide comprising the
nucleotide sequence substantially as set out in SEQ ID No: 2, or a variant or fragment
thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID
20 No: 2 is provided herein as SEQ ID No: 3, as follows:

AUGGGUGCGCAGGUGAGCCGUCAGAACGUGGGCACCCAUAGCACCCAGAACAUGGUGAGCAACGGCAGCA
GCCUGAACUAUUUCAACAUAACUAUUUCAAGAUUGCGGCGAGCAGCGGUGCGAGCCGUCUGGAUUUCAG
CCAGGAUCCGAGCAAAUUUACCGAUCCGGUGAAAGAUUGUGUGGAAAAAGGCAUUCGACCCUGCAGAGC
25 CCGAGCGUGGAAGCGUGCGGCUAUAGCGAUUCGUUAUUAUUCAGAUUACCCGUGGCGAUAGCACCAUACCA
GCCAGGAUGUGGCGAACGCGGUGGUGGGCUAUGGUGUGUGGGCCGAUUUUCUACCCCGCAGGAUGCGAC
CGCGAUUGAUAAACCGACCCAGCCGGAUACAGCAGCAACCGUUUUUAUACCCUGGAUAGCAAAUUGUGG
AACAGCACCAGCAAAGGUUGGUGGUGGAAACUGCCGGAUGCGCUGAAAGAUUUGGGCAUUUUUCGGCGAAA
ACAUGUUCUAUUAUUUCUGGGCCGUAGCGGCUAUACCGUGCAUGUGCAGUGCAACGCGAGCAAAUUUCA
30 UCAGGGCACCCUGCGUUGUGAUUAUCCGGAACAUACGUGGCCACCGUGAACAAAGGCAACGUGAAC
GCGGGCUAUAUUUAUACCCAUCCGGGCGAAGCGGGUCGUGAAGUGGGCACCCAGGUGGAAACGAAAAAC
AGCCGAGCGAUGAUAACUGGCGUAACUUUGAUGGCACCCUGCGGGCAACCGUGUAUUUUUCGCAUCA
GUUCAUUAACCGUGCGUAGCAACAACAGCGCGACCCUGAUUUGCGGUAUGUGAACCGGGUGCCGAUGGAU
AGCAUGGUGCGUCAUAACAUAUUGGAGCCUGGUGAUUAUUCGGUGUGCCAGCUGCAGAGCAACAACAUUA
35 GCAACAUUGUGCCGAUUACCGUGAGCAUUAAGCCCGAUGUGCGCGGAUUUAGCGGUGCGCGUGCGAAAAAC
CGUGGUGCAG

[SEQ ID No: 3]

Accordingly, in one embodiment, the immunogenic composition comprises an isolated
40 polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in
SEQ ID No: 3, or a variant or fragment thereof.

In one embodiment, the amino acid sequence of the RV-Bo6 VPo peptide is provided
herein as SEQ ID No: 4, as follows:

45

- 10 -

MGAQVSTQKSGSHENQNILTNNGSNQFTTVINYKDAASSSSAGQSFSTDPKFTPEVKDLMLKGAPALNS
 PNVEACGYSDRVQQITLGNSTITTQEAANAVVCYAEWPEYLPDGDASDVNKTSPKPTSVCRFYTLSEKWTW
 QNSSKGWCWKLPAKLDKMGVFGQNMFFHSLGRSGYTIHVQCNAKTFHSGCLLVVVIPEHQLASHKGGNV
 VKYKYTHPGESGIDLSTQLDEDEGPVKDPVYSMNGTLLGNLLIFPHQFINLRNTNTATIVVPYINSVPIDS
 5 MTRHNNVSLLLVPIAPLVAPTGSPQTLPTVTIAPMCTEFTGIRSKTIVPQ

[SEQ ID No: 4]

Accordingly, preferably the RV-Bo6 VPo peptide comprises an amino acid sequence substantially as set out in SEQ ID No: 4, or a variant or fragment thereof.

10

The VP4 peptide of RV-Bo6 constitutes amino acid residues 1-69 of SEQ ID No: 4 and the VP2 peptide of RV-Bo6 constitutes amino acid residues 70-331 of SEQ ID No: 4. Thus, in embodiments in which the immunogenic composition comprises a fragment of RV-Bo6 VPo, the fragment may comprise or consist of the amino acids forming the VP2
 15 and/or VP4 peptides.

In one embodiment, the RV-Bo6 VPo peptide is encoded by the nucleotide sequence (DNA) of SEQ ID No: 5, as follows:

20 ATGGGCGCTCAAGTTTCAACACAAAAGAGTGGTTCTCACGAGAATCAGAACATCTTAACTAATGGCTCAA
 ATCAGACGTTTACAGTTATAAAATATTATAAAGATGCAGCTAGCTCATCAGCAGGTCACTCTTCTC
 AACGGACCCATCAAAATTCACAGAACCAGTTAAAGACCTGATGTTGAAAGGTGCCCCAGCATTGAACCA
 CCAAATGTTGAAGCATGTGGTTATAGTGACAGAGTCCAACAAATTAATCTAGGTAATTCACATATAACAA
 CCCAGGAAGCAGCCAATGCGGTTGTGTGTATGCTGAGTGGCCTGAATACTTGCCAGATGGTGATGCTAG
 25 TGATGTTAATAAGACTTCAAACCAGACACATCTGTATGTAGATTCTACACATTGGAGAGTAAAACATGG
 CAAAACAGCTCAAAGGGATGGTGTGGAACTACCAGATGCTCTCAAGGATATGGGTGTGTTGGGCAAA
 ACATGTTCTTCCACTCGCTTGGTAGATCTGGCTATACTATACATGTGCAATGTAATGCAACTAAATCCA
 TAGTGGCTGTCTTCTTGTGTGTATCCAGAACACCAATTGGCATCTCACAAAGGCGGGAATGTGTCA
 GTGAAGTATAAATATACACATCCAGGAGAGAGTGGTATAGACTTGAGTACACAGTTAGATGAGGATGGTC
 30 CAGTGAAAGATCCTGTCTATAGCATGAATGGCACTCTATTAGGTAACCTACTCATCTTCCCTCACCAGTT
 TATCAACCTCAGAACAAACATACAGCCACAATAGTTGTGCCATATATTAATTCAGTGCCCAATAGACTCA
 ATGACACGCCATAATAACGTGTCACTCTTAGTGATGCCATAGCACCTCTAGTGGCTCCAAGTGGATCAC
 CACAAACACTTCCAGTGACAGTGACTATAGCTCCCATGTGTACAGAGTTACAGGGTATTAGATCAAAAAC
 CATTGTTCCACAG
 35

[SEQ ID No: 5]

Accordingly, preferably the RV-Bo6 VPo peptide is encoded by the nucleotide sequence substantially as set out in SEQ ID No: 5, or a variant or fragment thereof. Alternatively, the immunogenic composition comprises an isolated polynucleotide comprising the
 40 nucleotide sequence substantially as set out in SEQ ID No: 5, or a variant or fragment thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID No: 5 is provided herein as SEQ ID No: 6, as follows:

- 11 -

AUGGGCGCUCAAGUUUCAAACACAAAAGAGUGGUUCUCACGAGAAUCAGAACAUCUUAACUAAUGGCUCAA
 AUCAGACGUUCACAGUUUAAAUAUUAUAAAGAUAGCAGCUAGCUCUACUACAGCAGGUCAGUCUUUCUC
 AACGGACCCAUCAAAAUUCACAGAACCAGUUAAAGACCUGAUGUUGAAAGGUGCCCCAGCAUUGAACUCA
 5 CCAAAUGUUUGAAGCAUGUGGUUAUAGUGACAGAGUCCAACAAAUUACUCUAGGUAAUUCACUAUAACAA
 CCCAGGAAGCAGCCAAUGCGGUUGUGUGUUUAUGCUGAGUGGCCUGAAUACUUGCCAGAUUGGUGAUGCUAG
 UGAUGUUAAUAAGACUUCCAAACCAGACACAUCUGUAUGUAGAUUCUACACAUUGGAGAGUAAAACAUGG
 CAAAACAGCUCAAAGGGAUGGUGUUGGAAACUACCAGAUGCUCUCAAGGAUAUGGGUGUGUUUGGCGAAA
 ACAUGUUCUCCACUCGCUUGGUAGAUUCGGCUAUACUAUACAUGUGCAAUGUAAUGCAACUAAAUCCA
 10 UAGUGGCUGUCUUUUUGUUGUUGUUAUCCAGAACCAAUUGGCAUCUCACAAAGGCGGGAUUGUGUCA
 GUGAAGUAAUAAUUAACAUCCAGGAGAGAGUGGUUAUAGACUUGAGUACACAGUUAGAUGAGGAUGGUC
 CAGUGAAAGAUCCUGUCUAUAGCAUGAAUGGCACUCUAUUAAGGUAAACUUAUCUACUUCUCCUCACAGUU
 UAUCAACCUCAGAACAAACAAUACAGCCACAAUAGUUGUGCCAUUAUUAUUAUUCAGUGCCAAUAGACUCA
 AUGACACGCCAUAAUAACGUGUCACUCUUGAGUAUUCUCCAUAGCACCUCUAGUGGCUCCAACUGGAUCAC
 15 CACAAACACUUCAGUGACAGUGACUAUAGCUCUCCAUUGUGUACAGAGUUCACGGGUAAUUGAUCAAAAAC
 CAUUGUUCACAG

[SEQ ID No: 6]

Accordingly, in one embodiment, the immunogenic composition comprises an isolated
 20 polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in
 SEQ ID No: 6, or a variant or fragment thereof.

In one embodiment, the amino acid sequence of the RV-Co7 VPo peptide is provided
 herein as SEQ ID No: 7, as follows:

25 MGAQVSKQSVGAHETMVHAGSGAVVKYFNINYKDAASSGLTKQDFSQDPSKFTQPVADLLTNPALMSPS
 VEACGYSDRLKQITIGSSTITTQDSVNTIVAYGEWPSYLSDDLASSVDKPTHPETSADREFYTLDSVRWGG
 SSKGWWWKLPDCLKNMGI FGQNMYYHAMGRSGYIIHTQCNA TKFHS GCLLVAVVPEHQLAYIGGTNAQVS
 YKHTHPGERGHEIGLNTSRGDNRPDEDPFFNCNGTLLGNLTI FPHQLINLRTNNSSTIVVPYINCTPMD S
 30 MLRHNNVSLV IIPICPLRTPSGAPTTLPI TVSIAPDRSEFSGARQSATRQ

[SEQ ID No: 7]

Accordingly, preferably the RV-Co7 VPo peptide comprises an amino acid sequence
 substantially as set out in SEQ ID No: 7, or a variant or fragment thereof.

35 The VP4 peptide of RV-Co7 constitutes amino acid residues 1-67 of SEQ ID No: 7 and
 the VP2 peptide of RV-Co7 constitutes amino acid residues 68-330 of SEQ ID No: 7.
 Thus, in embodiments in which the immunogenic composition comprises a fragment of
 RV-Co7 VPo, the fragment may comprise or consist of the amino acids forming the VP2
 40 and/or VP4 peptides.

In one embodiment, the RV-Co7 VPo peptide is encoded by the nucleotide sequence
 (DNA) of SEQ ID No: 8, as follows:

- 12 -

ATGGGCGCTCAGGTTTCCAAGCAGAGTGTGGGAGCTCATGAACTATGGTGCATGCTGGCTCTGGTGCAG
TTGTTAAATACTTCAACATCAATTATTACAAGGATGCTGCTAGCTCCGGTTTGACCAAAACAAGATTCTC
ACAAGACCCATCCAAGTTTACTCAGCCTGTAGCAGATTACTGACAAATCCAGCTTTGATGTCCCATCA
GTTGAGGCGTGTGGATACTCCGATAGGCTCAAGCAAATCACTATCGGAAGCTCCACCATTACAACACAAG
5 ATTCTGTCAACACCATAGTTGCCTATGGCAGTGGCCTAGTTATTTGTGATTTAGATGCATCATCTGT
TGATAAACCCACACATCCTGAAACATCGGCTGACAGATTCTACACTTTGGACAGCGTACGCTGGGGTGGT
AGTTCCAAAGGATGGTGGTGGAGTTGCCAGACTGTTGAAGAACATGGGTATATTTGGGCAGAACATGT
ACTACCACGCTATGGGAAGGCTTGGTTACATCATAACACCCAGTGCATGCCACTAAGTTCCACAGTGG
ATGCTCTGTTGGTAGCGGTTGTTCCGAACACCAGCTAGCTTACATTGGGGGTACCAATGCACAAGTTAGC
10 TACAAACATACTCACCAGGTGAACGTGGGCATGAAATAGGTCTCAACACAAGCAGAGGTGACAACCGGC
CAGATGAGGATCCCTTTTAAATTGCAACGGAACACTATTAGGTAACCTGACTATTTTCCCTCACCAGCT
CATAAACCTGAGGACAAATAACTCCAGCACCATAGTTGTGCCTTACATTAACCTGCACACCAATGGACAGT
ATGTTAGGCCACAACAATGTCTCTTTGTAATTATACCCATCTGTCTTTACGAACTCCAGTGGAGCAC
CCACTACCTTACCAATTACTGTTTCAATTGCCCCAGACAGATCTGAATTCTCTGGCGCCAGGCAGTCCGC
15 CACCCGCCAG

[SEQ ID No: 8]

Accordingly, preferably the RV-Co7 VPO peptide is encoded by the nucleotide sequence
substantially as set out in SEQ ID No: 8, or a variant or fragment thereof. Alternatively,
20 the immunogenic composition comprises an isolated polynucleotide comprising the
nucleotide sequence substantially as set out in SEQ ID No: 8, or a variant or fragment
thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID
25 No: 8 is provided herein as SEQ ID No: 9, as follows:

AUGGGCGCUCAGGUUUCCAAGCAGAGUGUGGGAGCUC AUGAAACUAUGGUGCAUGCUGGCUCUGGUGCAG
UUGUUAAUACUUAACAUCUAUUAUUAACAAGGAUGCUGCUGAGCUCGGUUUGACCAAAACAAGAUUUCUC
ACAAGACCCAUCCAAGUUUACUCAGCCUGUAGCAGAUUUACUGACAAAUCCAGCUUUGAUGUCCCCAUCA
30 GUUGAGGCGUGUGGAUACUCCGAUAGGCUAAGCAAUACUAUCGGAAGCUCACCAUUAACAACAAG
AUUCUGUCAACACCAUAGUUGCCUAGUGGCAGUGGCCUAGUUUUUUGUCAGAUUUAGAUCAUCUCUGU
UGAUAAACCCACAUCCUGAAACAU CGGCU GACAGAUUUCUACAUUU GGACAGCGUACGUCUGGGUGGU
AGUUCCAAAGGAUGGUGGUGGAAGUUGCCAGACUGUUUGAAGAAUAUGGGUAUUAUUGGGCAGAAACUUGU
ACUACCACGCUAUGGGAAGGUCUGGUUACAUCAUACACACCCAGUGCAAUGCCACUAAGUUCACAGUGG
35 AUGUCUGUUGGUGAGCGGUUGUUCGGAACACCAGCUGCUUACAUAUUGGGGUACCAAUGCACAAGUUAGC
UACAAACAUACUACCCAGGUGAACGUGGCAUGAAAUAGGUCUACACACAAGCAGAGGUGACAACCGGC
CAGAUAGGAUCCCUUUUUUAUUGCAACGGAACACUAUUAAGGUAACUUGACUAUUUCCCUACACAGCU
CAUAAACUGAGGACAAUAACUCCAGCACCAUAGUUGGCCUUACAUAACUGCACACCAUUGGACAGU
AUGUUAACGCCACAACAAGUCUCUCUUGUAUUUAUACCCAUCUGUCCUUUACGAACUCCAGUGGAGCAC
40 CCACUACCUUACCAAUACUGUUUCAAUUGCCCCAGACAGAUUGAAUUCUCUGGCGCCAGGCAGUCCGC
CACCCGCCAG

[SEQ ID No: 9]

Accordingly, in one embodiment, the immunogenic composition comprises an isolated
45 polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in
SEQ ID No: 9, or a variant or fragment thereof.

- 13 -

In one embodiment, the amino acid sequence of the RV-C19 VPo peptide is provided herein as SEQ ID No: 10, as follows:

5 MGAQVSKQNVGSHENSVSASNGSVIKYFNINYYKDSASSGLTKQDFSQDPSKFTQPLVDLTNPALMSPS
VEACGFSDRLKQITIGNSTITTTQDSLNTVLAYGEWPSYLSDLDATSVDKPSHPETSSDRFYTLESVDWKS
GSKGWWWKFPDALKDMGMFGQNMYYHSMGRFGALIHVQCNAKFKHSGCLLIMVVEPHQLAYIGAEGVKVR
YETHHPGEKGHTLKGSQDRSTNNPDDNPFYMCNGTLLGNGLYPHQMINLRNNSATIVIPYINCVPMND
MLRHNNFSLVIVFIVPLRPGNSGAPILPITVTIAPYKSEFSGAMKSQPNQ [SEQ ID No: 10]

10

Accordingly, preferably the RV-C19 VPo peptide comprises an amino acid sequence substantially as set out in SEQ ID No: 10, or a variant or fragment thereof.

15 The VP4 peptide of RV-C19 constitutes amino acid residues 1-67 of SEQ ID No: 10 and the VP2 peptide of RV-C19 constitutes amino acid residues 68-330 of SEQ ID No: 10. Thus, in embodiments in which the immunogenic composition comprises a fragment of RV-C19 VPo, the fragment may comprise or consist of the amino acids forming the VP2 and/or VP4 peptides.

20 In one embodiment, the RV-C19 VPo peptide is encoded by the nucleotide sequence (DNA) of SEQ ID No: 11, as follows:

25 ATGGGTGCACAGGTCAGCAAACAGAATGTTGGATCACACGAAAACCTCTGTGTCTGCTAGTAATGGCTCTG
TAATCAAGTACTTTAACATCAATTACTATAAAGACTCTGCCAGCTCTGGGTGACTAAACAAGACTTTTC
ACAAGACCCGTCAAAATTTACTCAACCACTCGTTGACACACTGACAAATCCCGCCTTGATGTCCCCAAGT
GTTGAAGCTTGTGGGTTTTCCGATAGGCTTAAACAAATCACTATCGGAAACTCCACTATACACACAAG
ATTCGCTCAACACCGCTCTTGGCGTATGGAGAATGGCCTTCATACCTCTCTGACCTAGATGCCACCTCAGT
AGATAAACCATCACACCCAGAAACCTCTCAGACCGATTCTATACACTAGAAAGTGTGATTGGAAGTCC
GGATCTAAAGGTTGGTGGTGGAAATTTCCAGATGCCCTAAAAGATATGGGAATGTTTGGGCAAAACATGT
30 ACCACCATTCATGGGTAGGTTTGGAGCTCTCATACATGTCCAATGTAATGCTACTAAATTTACAGTGG
TTGTCTCCTGATCATGGTTGTCCCCGAACATCAATTAGCATATATAGGGGCAGAAGGTGTCAAGGTTAGA
TATGAGCACACACCCAGGAGAAAAGGCCATACCTTGAAAGGTAGCCAGGATAGATCAACTAACAAATC
CAGATGATAACCCATTCTACATGTGTAAACGGTACACTACTGGGTAATGGTATGTTATATCCTCATCAGAT
GATCAACCTCCGCACCAACAACCTCAGCCACTATTGTAATTCATACATCAACTGTGTACCCATGGACAAT
35 ATGTTGCGCCACAACAACCTTCAGTCTGGTGATTGTACCAATAGTACCTTGAGACCTGGGAACTCAGGAG
CTCCTATCTTACCCATCACTGTGACCATAGCACCATACAAATCAGAGTTTCTGGAGCTATGAAATCCCA
ACCCAATCAG [SEQ ID No: 11]

40 Accordingly, preferably the RV-C19 VPo peptide is encoded by the nucleotide sequence substantially as set out in SEQ ID No: 11, or a variant or fragment thereof. Alternatively, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence substantially as set out in SEQ ID No: 11, or a variant or fragment thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID No: 11 is provided herein as SEQ ID No: 12, as follows:

5 AUGGGUGCACAGGUCAGCAAACAGAAUGUUGGAUCACACGAAAACUCUGUGUCUGCUAGUAAUGGCUCUG
 UAAUCAAGUACUUUAAUCAUAAUACUUAUAAAGACUCUGCCAGCUCUGGGUUGACUAAACAGACUUUUC
 ACAAGACCCGUCAAAUUUACUCAACCACUCGUUGACACACUGACAAAUCCGCCUUGAUGUCCCAAGU
 GUUGAAGCUUGUGGGUUUCCGAUAGGCUUAAACAAUACUAUCGGAACUCCACUAUUACAACACAAG
 AUUCGCUCAACACCGUCUUGGCGUAUGGAGAAUGGCCUUCUAACUCUCUGACCUAGAUGCCACCUCAGU
 10 AGAUAACCAUCACACCCAGAAACCUCCUAGACCGAUUCUAUACACUAGAAAGUGUUGAUGGAAGUCC
 GGAUCUAAAGGUUGGUGGUGGAAUUUCCAGAUGCCUAAAAGAUUUGGGAUUGUUGGGCAAAACAUUGU
 ACCACCAUUCUAAUGGGUAGGUUUGGAGCUCUAUACAUUGUCCAAUGUAAUGCUACUAAAUUUCACAGUGG
 UUGUCUCCUGAUCAUGGUUGUCCCGAACAUCAAUAGCAUUAUAGGGGCAGAAGGUGUCAAGGUUAGA
 UAUGAGCACACACACCCAGGAGAAAAGGCCAUACCUUGAAAGGUAGCCAGGAUAGAUCAACUAAACAAUC
 15 CAGAUGAUAACCAUUCUACAUGUGUAACGGUACACUACUGGGUAAUGGUUUAUUAUCCUACUAGAU
 GAUCAACCUCCGACCAACAACUCAGCCACUAUUGUAAUCCAUACAUCAACUGUGUACCCAUGGACAAU
 AUGUUGCGCCACAACAACUUCAGUCUGGUUAUUGUACCAUAGUACCCUUGAGACCUGGGAACUCAGGAG
 CUCCUACUUAACCAUCACUGUGACCAUAGCACCACUACAAUCAGAGUUUUCUGGAGCUAUGAAAUCCCA
 ACCCAAUUACAG

[SEQ ID No: 12]

Accordingly, in one embodiment, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in SEQ ID No: 12, or a variant or fragment thereof.

25

In one embodiment, the amino acid sequence of the RV-C24 VPo peptide is provided herein as SEQ ID No: 13, as follows:

30 MGAQVSKQNVGSHENSVSATGGSVIKYFNINYYKDSASSGLTKQDFSQDPSKFTQPLAEALTNPALMSPT
 VEACGMSDRKQITIGNSTITTQDTLNSILAYGEWPKYLSDLDAASSVDKPTHPETSSDRFYTLTSVDWTT
 TSKGWWWKLPDCLKDMGIFGQNLHYHALGRSGYIIHTQCNA TKFN SGCLIVAVVPEHQ LAYIGEANVNVG
 YDHTHPGEGGHVIGSNVRRDNKQPDDEPFENCNGTLLGNITIFPHQLINLRTNNSSTIVVPYINCVPM DN
 MLRHNNLSLVIPIVPLRAANGVT KVPITIS IAPDKSEFSGARQSVKQ

[SEQ ID No: 13]

35

Accordingly, preferably the RV-C24 VPo peptide comprises an amino acid sequence substantially as set out in SEQ ID No: 13, or a variant or fragment thereof.

40 The VP4 peptide of RV-C24 constitutes amino acid residues 1-67 of SEQ ID No: 13 and the VP2 peptide of RV-C24 constitutes amino acid residues 68-328 of SEQ ID No: 13. Thus, in embodiments in which the immunogenic composition comprises a fragment of RV-C24 VPo, the fragment may comprise or consist of the amino acids forming the VP2 and/or VP4 peptides.

- 15 -

In one embodiment, the RV-C24 VPO peptide is encoded by the nucleotide sequence (DNA) of SEQ ID No: 14, as follows:

5 ATGGGCGCACAGGTCAGCAAGCAAAATGTCGGCTCGCATGAAAACCTCAGTCTCAGCCACAGGTGGATCCG
TGATTAAGTATTTCAACATCAATTACTACAAGGATCTGCTAGCTCTGGCTTGACTAAACAAGATTTTTC
CCAAGACCCATCGAAATTCACACAACCTCTAGCAGAAGCACTTACAAATCCAGCTTTAATGTCACCAACT
GTTGAAGCATGTGGGATGTCCGATAGGCTTAAACAAATTACTATCGGGAATTCCTACTATAACAACACAAG
ATACACTAAACTCTATACTGGCATATGGGGAGTGGCCCAAATACCTTGGAGTACCTGGACGCTTCCTCAGT
GGATAAACCTACCCACCCAGAAACATCATCTGATAGATTTTACACATTAAGTAGTGTAGATTGGACCACT
10 ACGTCTAAAGGTTGGTGGTGGAAAGTTGCCTGATTGCCTTAAAGATATGGGCATCTTCGGGCAAAATCTGT
ACCATCATGCATTTGGGTAGGTCAGGGTACATAATACACACCCCAATGTAATGCCACAAAATTCATAGTGG
TTGTCTAATAGTGGCTGTTGTACCAGAACCAGCTAGCTTACATAGGTGAAGCAAAATGTCAATGTTGGT
TATGATCACACACACCTTGGTGAGGGAGGACATGTAATTGGTTCAAATGTTAGGAGAGATAACAAGCAAC
CTGATGAAGACCCCTTCTTTAATTGTAATGGGACCCCTGCTTGGTAACATCACATATATCCCACACCAGCT
15 CATAAAGTGGAGACAAACAATTCAGCACAATTGTTGTACCATACATTAATTGTGTACCTATGGACAAC
ATGCTCAGGCACAACAACCTATCTCTAGTTATTATTCCAATCGTTCCCTCTCAGAGCCGCAAAATGGTGTCA
CCAAGGTCCCCATTACAATCTCAATAGCACCAGATAAGTCAGAGTTCTCAGGGGCTAGACAGTCTGTAAA
ACAG

[SEQ ID No: 14]

20

Accordingly, preferably the RV-C24 VPO peptide is encoded by the nucleotide sequence substantially as set out in SEQ ID No: 14, or a variant or fragment thereof.

Alternatively, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence substantially as set out in SEQ ID No: 14, or a

25 variant or fragment thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID No: 14 is provided herein as SEQ ID No: 15, as follows:

30 AUGGGCGCACAGGUCAGCAAGCAAAAUGUCGGCUCGCAUGAAAACUCAGUCUCAGCCACAGGUGGAUCCG
UGAUUAAGUAUUUCAAUAUCAAUUAUACAAGGAUUCUGCUAGCUCUGGCUUGACUAAACAAGAUUUUUC
CCAAGACCCAU CGAAAUUACACACAACCUUAGCAGAAGCACUUACAAAUCCAGCUUUAAUGUCACCAACU
GUUGAAGCAUGUGGGAUGUCCGAUAGGCUUAAACAAAUUACUACGGGAAUUCACUAUAACAACACAAG
AUACACUAAACUUAUACUGGCAUUAUGGGAGUGGCCCAAUACUUGAGUGACCUGGACGUUCCUCAGU
35 GGAUAAACCUACCCACCCAGAAACAUCAUCGAUAGAUUUACACAUUAACUAGUGUAGAUUGGACCACU
ACGUCUAAAGGUUGGUGGUGGAAGUUGCCUGAUUGCCUUAAGAUUAGGGCAUCUUCGGGCAAAAUUCUGU
ACCAUUAUGCAUUGGGUAGGUCAGGGUACAUAAUACACACCCAAUGUAAUGCCACAAAUUCAAUAGUGG
UUGUCUAAUAGUGGCUUUGUACCAGAACACCAGCUAGCUUACAUAGGUGAAGCAAAUGUCAAUUGGU
UAUGAUCACACACACCCUGGUGAGGGAGGACAUGUAAUUGGUUCAAUUGUAGGAGAGAUAAACAAGCAAC
40 CUGAUGAAGACCCUUCUUUAAUUGUAAUUGGACCCUGCUUGGUAAACAUACUAUUAUCCACACCAGCU
CAUAAACUUGAGGACAAACAUAUCCAGCACAAUUGUUGUACCAUACAUAAUUGUUAUCCUUAUGGACAA
AUGCU CAGGCACAACAACCUAUUCUAGUUAUUAUCCAAUCGUUCCUCU CAGAGCCGCAAAUGGUGUCA
CCAAGGUCCCCAUUACAAUCUCAAUAGCACCAGAUAGUCAGAGUUCU CAGGGGCUAGACAGUCUGUAAA
ACAGCAG

45

[SEQ ID No: 15]

- 16 -

Accordingly, in one embodiment, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in SEQ ID No: 15, or a variant or fragment thereof.

- 5 In one embodiment, the amino acid sequence of the RV-Co1 VPo peptide is provided herein as SEQ ID No: 16, as follows:

10 MGAQVSRQSVGSHETMIHAGTGAVVKYFNVNYYKDAASSGLTKQDFSQDPSKFTQPVADILTNPALMSPS
VEACGFSDDLKQITIGNSTITTQDAVNTIVAYGEWPSYLSDDLATSVDKPTHPETSSDRFYTLRSVDWRG
GSKGWWWKLPDCLKDMGIFGQNMFFHAMGRSGYIIHTQCNATKFHSGCLLVAVVPEHQLAYIGADARVSY
EHTHPGERGHTIGSNADRNNHQPDENPFNCNGTLLGNLTI FPHQLINLRTNNSSTIVVPYINCTPMDSM
LRHNNVSLVITPICPLRPPTTGQQVLPTITISIAPIKSEFSGARQAIKQ [SEQ ID No: 16]

- 15 Accordingly, preferably the RV-Co1 VPo peptide comprises an amino acid sequence substantially as set out in SEQ ID No: 16, or a variant or fragment thereof.

The VP4 peptide of RV-Co1 constitutes amino acid residues 1-67 of SEQ ID No: 16 and the VP2 peptide of RV-Co1 constitutes amino acid residues 68-328 of SEQ ID No: 16.

- 20 Thus, in embodiments in which the immunogenic composition comprises a fragment of RV-Co1 VPo, the fragment may comprise or consist of the amino acids forming the VP2 and/or VP4 peptides.

- 25 In one embodiment, the RV-Co1 VPo peptide is encoded by the nucleotide sequence (DNA) of SEQ ID No: 17, as follows:

30 ATGGGCGCACAGGTGTCTAGACAGAGTGTCTGGGAGCCATGAAACCATGATCCATGCTGGGACTGGAGCCG
TAGTAAAGTACTTCAATGTCAATTATTACAAAGATGCAGCAAGCTCTGGATTGACCAAGCAAGATTTCTC
CCAGGATCCATCCAAATTTACTCAACCCGTGGCCGACATCCTGACCAACCCAGCTTTGATGTCCCCCTCC
GTTGAAGCTTGTGGGTTTCCGATAGGCTCAAGCAAATCAGTATTTGGAACTCCACTATAACAACCCAAG
ATGCAGTCAATACAATTTGTGGCATAACGGGAATGGCCAGTTATCTATCAGATTTGGATGCGACTTCAGT
TGATAAACCGACGCACCTGAGACATCATCAGACAGATTTTACACGCTGAGGAGTGTGACTGGCGAGGT
GGTTCAAAGGGTGGTGGTGGAACTGCCTGATTGTCTGAAAGACATGGGAATATTTGGGCAAAACATGT
TCCACCATGCCATGGGAAGATCAGGATACATCATAACACCCAGTGCAATGCAACTAAATTTACAGTGG
35 ATGTTTACTCGTGGCAGTAGTGCCTGAACACCAATTAGCATACATAGGTGCAGATGCACGAGTGAGTTAT
GAGCACACCCACCTGGTGAGCGAGGCCATACAATTTGGTAGCAACGCAGACAGGAATAACCATCAGCCAG
ATGAAAATCCTTTCTTCAACTGTAACGGGACTTTACTTGGAATTTGACTATCTTCCACACACAGCTTAT
AAATTTAAGGACAAACAACTCAAGCACCATCGTCGTGCCTTACATCAATTGCACTCCTATGGATAGCATG
TTACGGCACAACAAATGTATCATTGTATCATACCCATTTGCCCTTTACGGCCCCCAACCACAGGGCAGC
40 AAGTCTTACCGATCACCATTTCATTGCTCCCATAAATCAGAGTTTTCAGGGGCTAGGCAGGCCATTAA
ACAA

[SEQ ID No: 17]

- 17 -

Accordingly, preferably the RV-Co1 VPo peptide is encoded by the nucleotide sequence substantially as set out in SEQ ID No: 17, or a variant or fragment thereof.

Alternatively, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence substantially as set out in SEQ ID No: 17, or a
 5 variant or fragment thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID No: 17 is provided herein as SEQ ID No: 18, as follows:

10 AUGGGCGCACAGGUGUCUAGACAGAGUGUCGGGAGCCAUGAAACCAUGAUCCAUGCUGGGACUGGAGCCG
 UAGUAAAGUACUUCUAUGUCAAUUUAUACAAAGAUGCAGCAAGCUCUGGAUUGACCAAGCAAGAUUUCUC
 CCAGGAUCCAUCCAAUUAUACUACACCGUGGCCGACAUCCUGACCAACCCAGCUUUGAUGUCCCCUCC
 GUUGAAGCUUGUGGGUUUCCGAUAGGCUCAAGCAAUCACUAUUGGAAACUCCACUAUACAACCCAAG
 15 AUGCAGUCAAUACA AUUGUGGCAUACGGGAAUUGGCCAGUUAUCUAUCAGAUUUGGAUUGCGACUUCAGU
 UGAUAAACCGACGCACCCUGAGACAUCAUCAGACAGAUUUUACACGCUAGGAGUGUUGACUGGCCGAGGU
 GGUUCAAAAGGUGGUGGUGGAAACUGCCUGAUUGUCUGAAAGACAUGGGAAUUAUUGGGCAAAACAUGU
 UCCACCAUGCCAUGGGAAGAUCAAGAUACAUCAUACACACCCAGUGCAAUGCAACUAAAUUUCACAGUGG
 AUGUUUACUCGUGGCAGUAGUGCCUGAACACCAAUUAGCAUACAUAAGGUGCAGAUUGCAGUGAGUUU
 GAGCACACCCACCCUGGAGCGAGGCCAUACA AUUGGUAGCAACGCAGACAGGAUAACCAUCAGCCAG
 20 AUGAAAAUCCUUUCUACUGUAACGGGACUUUACUUGGAAAUUUGACUAUCUCCCCACACAGCUUUAU
 AAUUUUAAGGACAAACAACUCAAGCACCAUCGUCGUGCCUACAUCAAUUGCACUCCUAUGGAUAGCAUG
 UUAACGGCACAAUGUAUCACUUGUUUAUCAUACCAUUGCCCUUACGGCCCCCAACACAGGGCAGC
 AAGUCUUAACGAUACCAUUAUUAUUGCUCCAUAAAAUCAGAGUUUUCAGGGGCUAGGCAGGCCAUUAA
 ACAA
 25 [SEQ ID No: 18]

Accordingly, in one embodiment, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in
 30 SEQ ID No: 18, or a variant or fragment thereof.

In one embodiment, the amino acid sequence of the RV-A28 VPo peptide is provided herein as SEQ ID No: 19, as follows:

35 MGAQVSRQNVGTHTTQNAVSNSSLNIFYFNINYFKDAASSGASRLDFSQDPSKFTDPVKDVLTKGIPTLQS
 PSVEACGYSDRIIQITRGNSTITSQDIANAVVAYGVWPEYLPQDASAIKPTHTPDTSSNRFYTLKSNW
 TATSKGWWWKLPDALKNMGIFGENLFYHFLGRAGYTVHVQCNAKFKHQGT LIVAAIPEHQLAFISKGNVT
 VGYNYTHPGEQGREIGTFGPSSTLDRQPSDENWLNCDGTLGNITVYPHQYINLRSNNSATLILPYVNAV
 PMDSMVRHNNWSIVIIPIISKLETEGGTTIVPITLSISPMFSEFSGARNNPQ
 40 [SEQ ID No: 19]

Accordingly, preferably the RV-A28 VPo peptide comprises an amino acid sequence substantially as set out in SEQ ID No: 19, or a variant or fragment thereof.

- 18 -

The VP4 peptide of RV-A28 constitutes amino acid residues 1-69 of SEQ ID No: 19 and the VP2 peptide of RV-A28 constitutes amino acid residues 70-332 of SEQ ID No: 19.

Thus, in embodiments in which the immunogenic composition comprises a fragment of RV-A28 VPo, the fragment may comprise or consist of the amino acids forming the VP2
5 and/or VP4 peptides.

In one embodiment, the RV-A28 VPo peptide is encoded by the nucleotide sequence (DNA) of SEQ ID No: 20, as follows:

10 ATGGGCGCTCAAGTATCTAGGCAGAATGTTGGCACACACACCACACAAAATGCTGTATCAAATGGCTCTA
GTTTGAATTATTTTAACATCAACTACTTTAAAGATGCTGCCCTCAAGTGGAGCATCACGACTAGATTTTTC
ACAAGATCCTAGTAAATTCAGTATCCAGTTAAAGATGTTCTTACAAAAGGTATACCAACTCTACAATCA
CCCAGTGTGAGGCATGTGGTTATTCAGATAGGATCATACAAATAACACGAGGGAATTCAACAATTACTT
15 CACAAGATATAGCAAATGCTGTAGTAGCCTATGGAGTTTGGCCTGAATATCTGCCTAGTCAAGATGCTAG
TGCAATAGACAAACCAACATCCTGACACCTCTTCTAACAGATTCTATACATTGGAAAGCAAAAATTGG
ACTGCAACCTCAAAGGATGGTGGTGAAACTTCCAGATGCTCTCAAGAATATGGGGATCTTTGGTGAGA
ATCTTTTCTATCATTTTCTTGGTAGAGCCGGGTACACTGTACATGTGCAGTGTAAATGCTAGCAAGTTTCA
CCAGGGTACCTAATAGTTGCTGCCATTCCAGAACATCAGTTGGCTTTCATTTCCAAGGGAATGTCACA
20 GTTGGTTATAATTATACACACCCCTGGTGAACAAGGCCGTGAAATAGGCACCTTTGGACCCAGTAGCACAT
TGGACAGACAACCTAGTGATGAAAATTGGCTTAATTGTGATGGCACACTTTTGGGAAATATAACAGTGTA
TCCACACCAATATATCAACCTCAGAAGTAACAATTAGCAACATTAATATTACCATATGTTAACGCCGTA
CCCATGGACTCTATGGTTAGGCATAACAATTGGAGTATAGTGATTATACCCATATCTAAATTAGAGACTG
AGGGTGGGACAAACAATTGTACCCATAACTTATCTATTAGCCCAATGTTTTCAGAGTTTCTGGGGCGAG
25 AAACAATGTCCCTCAA

[SEQ ID No: 20]

Accordingly, preferably the RV-A28 VPo peptide is encoded by the nucleotide sequence substantially as set out in SEQ ID No: 20, or a variant or fragment thereof.

Alternatively, the immunogenic composition comprises an isolated polynucleotide
30 comprising the nucleotide sequence substantially as set out in SEQ ID No: 20, or a variant or fragment thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID No: 20 is provided herein as SEQ ID No: 21, as follows:

35 AUGGGCGCUCAAGUAUCUAGGCAGAAUGUUGGCACACACACCACACAAAUGCUGUAUCAAUUGGCUCUA
GUUUGAAUUUUUAACAUCAACUACUUUAAAGAUUGCUCUCAAGUGGAGCAUCACGACUAGAUUUUUC
ACAAGAUCCUAGUAAAUUCACUGAUCCAGUUAAGAUUUUACAAAAGGUUACCAACUCUACAAUCA
40 CCCAGUGUUGAGGCAUGUGGUUAUUCAGAUAGGAUCAUACAAUAAACACGAGGGAUUCAACAUAUCUU
CACAAGAUUAGCAAUUGCUAGUAGCCUAGGAGUUUGGCCUGAAUUAUCGCCUAGUCAAGAUUCUAG
UGCAAUAGACAAACACAUCCUGACACCUUUUUAACAGAUUCUUAUACAUUGGAAAGCAAAAUUGG
ACUGCAACCUCAAAAAGGAUGGUGUGGAAACUUCAGAUUCUUAAGAAUUAUGGGGAUCUUUGGUGAGA
AUCUUUUUAUACAUUUCCUUGGUAGAGCCGGGUACACUGUACAUUGCAGUGUAAUGCUAGCAAGUUUCA
45 CCAGGGUACCCUAAUAGUUGCUGCCAUUCCAGAACAUAGUUGGCUUUCAUUUCCAAGGGUAAUUGCACA
GUUGGUUAUAAUUAACACACCCUGGUGAACAGGCCGUGAAUAGGCACCUUUGGACCCAGUAGCAU
UGGACAGACAACCUAGUGAUGAAAAUUGGCUUAAUUGUGAUGGCACACUUUUGGAAAUUAACAGUGUA

UCCACACCAUAUAUCAACCCUCAGAAGUAACAAUUCAGCAACAUUAAUAUUACCAUAUGUUAAACGCCGUA
CCCAUGGACUCUAUGGUUAGGCAUAACAAUUGGAGUAUAGUGAUUAUACCAUAUCUAAAAUAGAGACUG
AGGGUGGGACAACAAUUGUACCAUAACUUAUUCUAUUAGCCCAUGUUUUCAGAGUUUUCUGGGGCGAG
AAACA AUGUCCUCA

5

[SEQ ID No: 21]

Accordingly, in one embodiment, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in SEQ ID No: 21, or a variant or fragment thereof.

10

In one embodiment, the amino acid sequence of the RV-A29 VPO peptide is provided herein as SEQ ID No: 22, as follows:

15

MGAQVSRQNVGTHSTQNSVNSGSSLNYNINFKDAASSGASKLEFSQDPSKFTDPVKDVLEKGIPTLQS
 PTVEACGYSDRIMQITRGDSTITSQDVANAVIGYGVWPHYLSAEDATAIDKPTQPDTSNRFYLTLESKTW
 NRQSKGWWWWKLPDALKDMGIFGENMYHYHLGRSGYTVHVQCNASKFHQGTLLVVMPEHQLASVGTDKVG
 PGYAFTHPGEAGRGIVNSEPTSKHPSDDNLDGTLLGNVLFPHQFINLRSNNNSATIIVPVYNAVPM
 DMSLRHNNWSLVIPISELQENATNVTVPITVSI SPMFAEFSGARARPARATTQ

[SEQ ID No: 22]

20

Accordingly, preferably the RV-A29 VPO peptide comprises an amino acid sequence substantially as set out in SEQ ID No: 22, or a variant or fragment thereof.

25 The VP4 peptide of RV-A29 constitutes amino acid residues 1-69 of SEQ ID No: 22 and the VP2 peptide of RV-A29 constitutes amino acid residues 70-335 of SEQ ID No: 22. Thus, in embodiments in which the immunogenic composition comprises a fragment of RV-A29 VP0, the fragment may comprise or consist of the amino acids forming the VP2 and/or VP4 peptides.

30 In one embodiment, the RV-A29 VP0 peptide is encoded by the nucleotide sequence (DNA) of SEQ ID No: 23, as follows:

ATGGGCGCTCAAGTATCCAGACAAAAATGTTGGAACATCTTCTACCCAAAATTTCAGTTTCAAATGGATCGA
GTTTAAATTATTTTAAACATAAATTACTTTAAGGATGCTGCATCAAGTGGAGCCTCAAACCTGGAATTTTC
35 TCAAGATCCTAGCAAATTCATGACCCGGTGAAAGATGTTTATAGAAAAAGGTATTCCAACACTACAAATCC
CCTACGGTGGAGGCATGTGGTTATTTCAGATCGGATAATGCAAATTACAAGAGGTGACTCCACAATAACAT
CTCAAGATGTGGCTAATGCAGTTATGAGTTATGAGATCTGGCCACCACTATCTGAGTGTGAAGATGCTAC
TGCTATTGATACAACTACCCAGCCAGACACCTCATCAAAATGAGTTTACACCTTGGAGAGTAAGACATGG
AATAGGCAATCCAAAGGATGGTGGTGAAATTGCCAGATGCACCTTAAAGATATGGGCATATTTGGAGAAA
40 ATATGTATTATCATTACCTTGGCAGGAGTGGTTATACAGTACATGTCCAATGCAATGCTAGTAAGTTTCA
TCAAGGAACATTACTGGTAGTGATGATACCTGAGCACCACCTGGCAAGTGTAGGTACTGATAAAGTCGGA
CCTGGATATGCTTTCACGCGATCCCGGTGAGGCAGGTAGACAGGATGGCAATGTGAGTGAACCGACTTCAA
AGCATCCAAGTGATGACAAATGGCTCAACTTTGATGGCACCCTTACTGGGTAATGTTCTTATATCTCCCA
CCAATTTATCAATTTAAGGAGCAATAAATCTGCAACAATAATAGTTCTTATGTGAATGCTGTCCCAATG

- 20 -

GATTCAATGTTAAGACATAATAATTGGAGTTTAGTGATAATTCCAATCAGTGAATTACAAGCAGAGAATG
CCACTAATGTACGGTCCTATTACTGTATCAATTAGTCCAATGTTTGCTGAATTTTCTGGAGCACGTGC
AAGGCCAGCAAGAGCTACTACACAG

[SEQ ID No: 23]

5

Accordingly, preferably the RV-A29 VPo peptide is encoded by the nucleotide sequence substantially as set out in SEQ ID No: 23, or a variant or fragment thereof.

Alternatively, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence substantially as set out in SEQ ID No: 23, or a

10 variant or fragment thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID No: 23 is provided herein as SEQ ID No: 24, as follows:

15 AUGGGCGCUCAAGUAUCCAGACAAAUGUUGGAACUCAUUCUACCCAAAUAUCAGUUUCAAUGGAUCGA
GUUUAAAUAUUUUUACAUAUUUUACUUUAAGGAUUCUGCAUCAAGUGGAGCCUAAAACUUGAAUUUUC
UCAAGAUCCUAGCAAAUUCACUGACCCGGUGAAAGAUUUUUAGAAAAAGGUUUUCCAACACUACAAUCC
CCUACGGUGGAGGCAUGUGGUUAUUCAGAUCCGAUAAUGCAAAUUAACAAGAGGUGACUCCACAUAACAUC
CUCAAGAUUGGCUAAUGCAGUUAUAGGUUAUUGGAGUCUGGCCACACUAUCUGAGUGCUGAAGAUUCUAC
20 UGCUAUUGAUAAACCUACCCAGCCAGACACCUCUAUCAAUAGAUUUUACACCUUGGAGAGUAAGACAUGG
AAUAGGCAAUCCAAAGGAUGGUGGUGGAAAUUGCCAGAUGCACUAAAGAUUAGGGCAUUAUUGGAGAAA
AUAUGUAUUUAUUAUACCUUGGCAGGAGUGGUUAUACAGUACAUGUCCAAUGCAAUGCUAGUAAGUUUCA
UCAAGGAACAUUACUGGUAGUGAUUAUACCUUGGAGCACAACUGGCAAGUGUAGGUACUGAUAAAGUCGGA
CCUGGAUUGCUUUACGCAUCCCGGUGAGGCGAGUAGACAGAUUGGCAAUGUGAGUGAACCGACUUCAA
25 AGCAUCCAAGUGAUGACAAUUGGCUAACUUUUGAUGGCACCUUACUGGGUAAUGUUUUUAUUAUCCCA
CCAUUUUUAUCAAUUUAAGGAGCAUAAUUCUGCAACAUAUUAUAGUUCCUUUUGUAAUUGCUUCCCAUG
GAUUCAAUGUUUAGACAUAUAAUUGGAGUUUAGUGAUAAUCCAAUCAGUGAAUUAACAAGCAGAGAAUG
CCACUAAUGUCACGGUCCCUAUUACUGUAUCAAUUAGUCCAAUGUUUGCUAAUUUUUCUGGAGCACGUGC
AAGGCCAGCAAGAGCUACUACACAG

30

[SEQ ID No: 24]

Accordingly, in one embodiment, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in

35 SEQ ID No: 24, or a variant or fragment thereof.

In one embodiment, the amino acid sequence of the RV-A89 VPo peptide is provided herein as SEQ ID No: 25, as follows:

40 MGAQVSRQNVGTHSTQNSVSNSSLNIFNINFKDAASSGASRLDFSQDPSKFTDPVKDVLEKGIPTLQS
PTVEACGYSDRLIQITRGDSTITSQDIANAVVAYGVWPSYLTDPDATAIDKPTQPDTSNRFYTLDSRSW
TSASSGWWWKLPDALKNMGI FGENMFYHFLGRSGYTIHVQCNSSKFHQGLLI VAAI PEHQLASATSGNVS
VGYNHTHPGEQGREVVPSRTSSDNKRPSDDSWLNF DGTLLGNLPIYPHQYINLRTNNSATLILPYVNAV
45 MDSMLRHHNWSLVIIPICLQVQPGGTQSIPITVSI SPMFSEFSGPRNKVVFSTTQ

[SEQ ID No: 25]

Accordingly, preferably the RV-A89 VPo peptide comprises an amino acid sequence substantially as set out in SEQ ID No: 25, or a variant or fragment thereof.

- 5 The VP4 peptide of RV-A89 constitutes amino acid residues 1-69 of SEQ ID No: 25 and the VP2 peptide of RV-A89 constitutes amino acid residues 70-336 of SEQ ID No: 25. Thus, in embodiments in which the immunogenic composition comprises a fragment of RV-A89 VPo, the fragment may comprise or consist of the amino acids forming the VP2 and/or VP4 peptides.

10

In one embodiment, the RV-A89 VPo peptide is encoded by the nucleotide sequence (DNA) of SEQ ID No: 26, as follows:

15 ATGGGTGCACAAGTATCTAGACAGAATGTTGGGACACACTCCACACAGAATTCAGTGAGCAATGGTTCTA
GTTTAAATTATTTCAACATCAATTATTTCAAGGATGCAGCCTCAAGTGGTGCTTCTAGATTAGATTTCTC
CCAAGATCCTAGTAAATTTCACTGACCCTGTAAAGATGTTTGTAGAAAAGGTATTCCTCAACTCCAGTCA
CCGACAGTTGAGGCTTGTGGTTATTCAGACAGGCTAATACAAATAACCCGAGGAGATTCCACTATAACGT
CTCAGGATATTGCAAATGCAGTTGTCGCTTATGGTGTGTTGGCCATCATACCTAACTCCAGATGATGCGAC
TGCCATTGACAAACCTACACAACCTGATACTTCATCCAATAGATTCTACACTCTGGATAGTCGCTCTTGG
20 ACATCTGCCTCATCTGGTTGGTGGTGGAAATTGCCCTGATGCCCTTAAGAACATGGGTATCTTTGGTGAAA
ACATGTTTTATCACTTTCTAGGGAGATCTGGGTACACAATACATGTACAGTGTAAATTTAGTAAGTTCCA
TCAGGTTTGTGATAGTTGCTGCTATCCCAGAGCATCAATTGGCATCTGCGACAAGTGGAAATGTATCA
GTTGGATATAACCCACCCACCCAGGTGAACAAGGTAGAGAAGTGGTGCCATCACGGACTTCTAGTGATA
ACAAAAGACCTAGTGATGATAGTTGGTTGAACCTTTGATGGGACATTACTTGGTAACCTGCCTATTTATCC
25 CCACCAATATATTAATTAAAGGACTAATAATTGAGCTACCTTATTTTACCTTATGTTAATGCTGTACCA
ATGGATTCTATGCTTAGACACAATAATTGGAGTTTGAATTATAATCCCAATATGCCCTCTTCAAGTTCAAC
CAGGAGGGACACAATCCATACCTATAACAGTATCAATCAGCCCTATGTTTTCAGAATTTTCAGGGCCAAG
GAATAAGGTTGTATTTAGTACCACTCAA

[SEQ ID No: 26]

30

Accordingly, preferably the RV-A89 VPo peptide is encoded by the nucleotide sequence substantially as set out in SEQ ID No: 26, or a variant or fragment thereof.

Alternatively, the immunogenic composition comprises an isolated polynucleotide comprising the nucleotide sequence substantially as set out in SEQ ID No: 26, or a

- 35 variant or fragment thereof.

In one embodiment, the RNA sequence corresponding to the DNA sequence of SEQ ID No: 26 is provided herein as SEQ ID No: 27, as follows:

40 AUGGGUGCACAAGUAUCUAGACAGAAUGUUGGGACACACUCCACACAGAAUUCAGUGAGCAAUGGUUCUA
GUUUAAAUAUUUCAACAUCAAUAUUUCAAGGAUGCAGCCUCAAGUGGUGCUUUCUAGAUUAGAUUUCUC
CCAAGAUCCUAGUAAAUCACUGACCCUGUUAAGAUUUUAGAAAAAGGUUUCACACUCCAGUCA
CCGACAGUUGAGGCUUGUGGUUAUUCAGACAGGCUAAUACAAUAACCCGAGGAGAUUCCACUAUAACGU
CUCAGGAUAUUGCAAAUGCAGUUGUCGCUUUGGUUUUGCCAUCUAUACCUAACUCCAGAUUGCGAC

- 22 -

UGCCAUUGACAAACCUACACAACCUGAUACUUAUCCAAUAGAUUCUACACUCUGGAUAGUCGCUCUUGG
 ACAUCUGCCUCAUCUGGUUGGUGGUGGAAAUUGCCUGAUGCCCUAAAGAACAUUGGUAUCUUGGUGAAA
 ACAUGUUUUUAUCACUUUCUAGGGAGAUUCUGGUACACAAUACAUGUACAGUGUAAUUCUAGUAAGUCCAA
 UCAGGGUUUGUUGAUAGUUGCUGCUAUCCAGAGCAUCAAUUGGCAUCUGCGACAAGUGGAAUUGUAUCA
 5 GUUGGAUAUAACACACCCACCCAGGUGAACAAAGGUAGAGAAGUGGUGCCAUACCGACUUCUAGUGAUA
 ACAAAGACCUAGUGAUGAUAGUUGGUUGAACUUUGAUGGGACAUUACUUGGUAACCUAGCCUAAUUUUAUCC
 CCACCAUAUAUUAAUUUAAGGACUAAUAAUUCAGCUACCCUUAUUUUACCUUAUGUUAAUGCUGUACCA
 AUGGAUUCUAGCUUAGACACAAUAAUUGGAGUUUAGUUUAUAAUCCCAUAUGCCUCUUCAGUUAAC
 CAGGAGGGACACAAUCCAUACCUAUAACAGUAUCAUACAGCCCUAUGUUUUCAGAAUUUUCAGGGCCAAG
 10 GAAUAAGGUUGUAUUUAGUACCACUCAA

[SEQ ID No: 27]

Accordingly, in one embodiment, the immunogenic composition comprises an isolated
 polynucleotide comprising the nucleotide sequence (RNA) substantially as set out in
 15 SEQ ID No: 27, or a variant or fragment thereof.

The term “immunogenic composition” as used throughout, refers to a composition of
 matter (intended to be administered to a subject) that comprises at least one antigen or
 induces the expression of at least one antigen of a rhinovirus (in the case of nucleic acid
 20 immunisation), which has the capability to elicit an immunological response in the
 subject to which it is administered. Such an immune response can be a cellular and/or
 antibody-mediated immune response directed at least against the antigen of the
 composition.

25 The immunogenic composition described herein provides an effective means of
 vaccinating a subject against RV infection. Accordingly, in a preferred embodiment, the
 immunogenic composition is a vaccine.

The immunogenic composition may comprise a suitable adjuvant. In a preferred
 30 embodiment, the adjuvant is a Th1 adjuvant. Examples of Th1 adjuvants promoting a
 Th1 immune response include TLR-9 agonists such as CpG oligonucleotides, or TLR-4
 agonists. Alternatively in another embodiment, the adjuvant is Incomplete Freund's
 Adjuvant (IFA).

35 Further examples of adjuvants may include an aluminium salt, a synthetic form of
 DNA, a carbohydrate, a tablet binder, an ion exchange resin, a preservative, a polymer,
 an emulsion and/or a lipid. Examples of adjuvants may include monosodium
 glutamate, sucrose, dextrose, aluminum bovine, human serum albumin, cytosine
 phosphoguanine, potassium phosphate, pladone C, anhydrous lactose, cellulose,
 40 polacrilin potassium, glycerine, asparagine, citric acid, potassium phosphate

- 23 -

magnesium sulfate, iron ammonium citrate, 2-phenoxyethanol, aluminium, beta-propiolactone, bovine extract, DOPC, EDTA, formaldehyde, thimerosal, phenol, potassium aluminum sulfate, potassium glutamate, sodium borate, sodium metabisulphite, urea, PLGA, PVA, PLA, PVP, cyclodextrin-based stabilisers, oil in water
5 emulsion adjuvants and/or lipid-based adjuvants.

The immunogenic composition according to the first aspect is particularly suitable for therapy or prophylaxis (i.e. vaccination) against rhinovirus infections.

10 Hence, in a second aspect of the invention, there is provided the immunogenic composition according to the first aspect, for use in therapy or prophylaxis.

In a third aspect, there is provided the immunogenic composition according to the first aspect, for use in eliciting an immune response.

15 In a fourth aspect, there is provided a method of eliciting an immune response in a subject, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of an immunogenic composition according to the first aspect.

20 It will be appreciated that the use and method of the invention comprises vaccination.

In a preferred embodiment, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, elicits an immune response
25 against a rhinovirus infection.

Preferably, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, elicits an immune response against at least one, at least two, or at least three species of rhinoviruses, more particularly, against RV-A,
30 RV-B, and/or RV-C.

Advantageously, the immune response that is induced by the immunogenic composition according to the first aspect, is a specific cell-mediated immune response not only directed to the homologous strain(s) of rhinovirus from which the
35 immunogenic composition is derived but also to other (heterologous) strains of

- 24 -

rhinoviruses of the same species of rhinoviruses, which can extend to strains of rhinoviruses of another group of rhinoviruses.

Preferably, therefore, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, elicits an immune response against at least one strain of RV-A, selected from a group consisting of: RV-1A, RV-A1, RV-A10, RV-A100, RV-A101, RV-A103, RV-A106, RV-A107, RV-A108, RV-A11, RV-A12, RV-A13, RV-A15, RV-A16, RV-A18, RV-A19, RV-A1B, RV-A2, RV-A20, RV-A21, RV-A22, RV-A23, RV-A24, RV-A25, RV-A28, RV-A29, RV-A30, RV-A31, RV-A32, RV-A33, RV-A34, RV-A36, RV-A38, RV-A39, RV-A40, RV-A41, RV-A43, RV-A44, RV-A45, RV-A46, RV-A47, RV-A49, RV-A50, RV-A51, RV-A53, RV-A54, RV-A55, RV-A56, RV-A57, RV-A58, RV-A59, RV-A60, RV-A61, RV-A62, RV-A63, RV-A64, RV-A65, RV-A66, RV-A67, RV-A68, RV-A7, RV-A71, RV-A73, RV-A74, RV-A75, RV-A76, RV-A77, RV-A78, RV-A8, RV-A80, RV-A81, RV-A82, RV-A85, RV-A88, RV-A89, RV-A9, RV-A90, RV-A94, RV-A95, RV-A96, and RV-A98. In a preferred embodiment, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, elicits an immune response against all strains of RV-A.

Preferably, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, elicits an immune response against at least one strain of RV-B, selected from a group consisting of: RV-B103, RV-B102, RV-B101, RV-B100, RV-B27, RV-B26, RV-B4, RV-B97, RV-B35, RV-B84, RV-B93, RV-B92, RV-B91, RV-B17, RV-B5, RV-B42, RV-B6, RV-B37, RV-B48, RV-B69, RV-B52, RV-B72, RV-B3, RV-B14, RV-B99, RV-B86, RV-B83, RV-B79, and RV-B70. In a preferred embodiment, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, elicits an immune response against all strains of RV-B.

Preferably, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, elicits an immune response against at least one strain of RV-C, selected from a group consisting of: RV-C58, RV-C50, RV-C48, RV-C46, RV-C13, RV-C51, RV-C55, RV-C56, RV-C45, RV-C44, RV-C41, RV-C4, RV-C38, RV-C34, RV-C33, RV-C31, RV-C30, RV-C29, RV-C27, RV-C26, RV-C24, RV-C23, RV-C21, RV-C16, RV-C14, RV-C47, RV-C53, RV-C54, RV-C22, RV-C37, RV-C49, RV-C25, RV-C18, RV-C12, RV-C20, RV-C19, RV-C28, RV-C36, RV-C39, RV-C15, RV-C5, RV-C11, RV-C9, RV-C10, RV-C1, RV-C43, RV-C42, RV-C40, RV-C32, RV-C17, RV-C8, RV-C7, RV-C6, RV-C3, RV-C2, and RV-C35. In a preferred embodiment, the immunogenic

- 25 -

composition for use according to the third aspect, or the method according to the fourth aspect, elicits an immune response against all strains of RV-C.

Furthermore, it is well-known that rhinoviruses are the major cause of common colds,
5 virus-induced wheezing illnesses in early childhood and acute attacks (exacerbations)
of lung diseases such as asthma, bronchiectasis, chronic obstructive pulmonary disease
(COPD), cystic fibrosis and chronic fibrosing lung diseases.

Accordingly, in a preferred embodiment, the immunogenic composition for use
10 according to the third aspect, or the method according to the fourth aspect, elicits an
immune response against common colds, virus-induced wheezing illnesses, acute
attacks of lung diseases such as asthma, exacerbations of asthma, bronchiectasis,
chronic obstructive pulmonary disease (COPD), cystic fibrosis and/or chronic fibrosing
lung disease.

15 As used herein, the expression “eliciting an immune response” may involve inducing a
specific cell-mediated immune response. This means the generation of a specific T
lymphocyte response following the administration of an immunogenic composition in a
subject. The two main cellular effectors of the specific T lymphocyte response are the
20 helper T-cells and the cytotoxic T lymphocytes (CTLs).

CD4+ “helper” T-cells or helper T-cells, are immune response mediators, and play an
important role in establishing and maximizing the capabilities of the adaptive immune
response. These cells can have to some extent a direct cytotoxic activity, but, in essence
25 “manage” the immune response, by directing other cells involved in the protection of
organisms against pathogens. The activation of a naive helper T-cell causes it to release
cytokines, which influences the activity of many cell types such as B lymphocytes, CTLs,
and APCs (Antigen Presenting Cells) that activated it. Helper T-cells require a much
milder activation stimulus than cytotoxic T-cells. Helper T-cells can provide extra
30 signals that “help” activate cytotoxic cells. Two types of effector CD4+ helper T cell
responses can be induced by a professional APC, designated Th1 and Th2. The measure
of cytokines associated with Th1 or Th2 responses will give a measure of successful
immunisation. This can be achieved by specific ELISA or ELISPOT designed for
measurement of Th1-cytokines such as IFN- γ , IL-2, and others, or Th2- cytokines such
35 as IL-4, IL-5, IL-13 among others.

- 26 -

As used herein, the expression “helper T-cell-mediated immune response” refers to an immune response wherein CD4⁺ T-cells or helper T-cells are activated and secrete lymphokines to stimulate both cell-mediated and antibody-mediated branches of the immune system. As known from the skilled person, helper T-cell activation promotes
5 lymphokine secretion, immunoglobulin isotype switching, affinity maturation of the antibody response, macrophage activation and/or enhanced activity of natural killer and cytotoxic T-cells. Lymphokines are proteins secreted by lymphocytes that affect their own activity and/or the activity of other cells. Lymphokines include, but are not limited to, interleukins and cytokines, *e.g.*, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, or IFN- γ .

10

It is well known to the skilled person that helper T-cells differentiate into two major subtypes of cells known as Th1 and Th2 cells (also known as Type 1 and Type 2 helper T cells, respectively).

15 Additionally, it is well known to the skilled person that Th1 cells mainly secrete IL-2 and IFN- γ . They promote cellular immune response by maximizing the killing efficacy of macrophages and the proliferation of cytotoxic CD8⁺ T-cells. Additionally, the type 1 cytokine IFN- γ increases the production of IL-12 by dendritic cells and macrophages, and, via positive feedback, IL-12 stimulates the production of IFN- γ in helper T-cells,
20 thereby promoting the Th1 profile. IFN- γ also inhibits the production of cytokines such as IL-4, an important cytokine associated with the Type 2 response, and thus it also acts to preserve its own response.

On the contrary, Th2 cells mainly secrete IL-4, IL-5 and IL-13, and promote humoral
25 immune response by stimulating B cells into proliferation, inducing B-cell antibody class switching. The Type 2 response further promotes its own profile using two different cytokines. IL-4 acts on helper T-cells to promote the production of Th2 cytokines (including itself), while IL-10 inhibits a variety of cytokines including IL-2 and IFN- γ in helper T-cells and IL-12 in dendritic cells and macrophages.

30

Preferably, the cell-mediated immune response induced by the immunogenic composition of the invention is primarily a Th1 cell-mediated immune response. In a preferred embodiment, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, induces an immune response
35 through the secretion of IFN- γ .

A critical component of protective cellular immunity elicited by natural infection with respiratory viruses is the formation of tissue resident memory T cells (T_{RM}) in the airway mucosa and lungs. These T_{RM} cells rapidly expand and clear subsequent
5 infections. Accordingly, in another preferred embodiment, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, induces an immune response through the formation of tissue resident memory T cells (T_{RM}).

10 In another preferred embodiment, the immunogenic composition for use according to the third aspect, or the method according to the fourth aspect, induces an immune response through the production of RV VP0-specific IgG antibodies.

Alternatively, in another embodiment, immunogenic composition for use according to
15 the third aspect, or the method according to the fourth aspect, induces a cytotoxic T cell immune response.

Cytotoxic T cells (also known as Tc, killer T cell, or cytotoxic T-lymphocyte (CTL)), which express generally the CD8 marker, are a sub-group of T cells and may also be
20 involved in the T cell-mediated immune response. They induce the death of cells that are infected with viruses (and other pathogens). These CTLs directly attack other cells carrying certain foreign or abnormal molecules on their surface. The ability of such cellular cytotoxicity can be detected using *in vitro* cytolytic assays (chromium release assay). Thus, induction of a specific cellular immunity can be demonstrated by the
25 presence of such cytotoxic T cells, when antigen-loaded target cells are lysed by specific CTLs that are generated *in vivo* following vaccination or infection.

Similarly to helper T-cells, CD8⁺ T-cells include distinct subsets, which were termed, analogously to the Th1/Th2 terminology, Tc1 and Tc2.

30 The Tc1 immune response involves specific IFN- γ -producing CD8⁺ T-cells which are activated, proliferate and produce IFN- γ upon specific antigen stimulation. The level of IFN- γ -producing CD8⁺ T-cells can be measured by ELISPOT and by flow cytometry measurement of intracellular IFN- γ in these cells.

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- 28 -

Naive cytotoxic T cells are activated when their T-cell receptor (TCR) strongly interacts with a peptide-bound MHC class I molecule. This affinity depends on the type and orientation of the antigen/MHC complex, and is what keeps the CTL and infected cell bound together. Once activated the CTL undergoes a process called clonal expansion in which it gains functionality, and divides rapidly, to produce an army of “armed” effector cells. Activated CTL will then travel throughout the body in search of cells bearing that unique MHC Class I + peptide. This could be used to identify such CTLs *in vitro* by using peptide-MHC Class I tetramers in flow cytometric assays.

When exposed to these infected cells, effector CTL release perforin and granzyme, cytotoxins which form pores in the target cell's plasma membrane, allowing ions and water to flow into the infected cell, and causing it to burst or lyse. CTL release granzyme, a serine protease that enters cells via pores to induce apoptosis (cell death). Release of these molecules from CTL can be used as a measure of successful induction of cellular immune response following vaccination. This can be done by enzyme linked immunosorbant assay (ELISA) or enzyme linked immunospot assay (ELISPOT) where CTLs can be quantitatively measured. Since CTLs are also capable of producing important cytokines such as IFN- γ , quantitative measurement of IFN- γ -producing CD8 cells can be achieved by ELISPOT and by flow cytometric measurement of intracellular IFN- γ in these cells.

It will be appreciated that the immunogenic composition according to the invention, may be used in a medicament, which may be used as a monotherapy (i.e. use of the immunogenic composition alone), for vaccination against an RV infection. Alternatively, the immunogenic composition according to the invention may be used as an adjunct to, or in combination with, known therapies for treating, ameliorating, or preventing an RV infection.

The immunogenic composition of the invention may be combined in compositions having a number of different forms depending, in particular, on the manner in which the composition is to be used. Thus, for example, the composition may be in the form of a powder, tablet, capsule, liquid, ointment, cream, gel, hydrogel, aerosol, spray, micellar solution, transdermal patch, liposome suspension, polyplex, emulsion, lipid nanoparticles (e.g. with peptide, DNA or RNA on the surface or encapsulated) or any other suitable form that may be administered to a person or animal in need of vaccination. The lipid nanoparticle may comprise one or more components selected

from a group consisting of: a cationic lipid (which is preferably ionisable); phosphatidylcholine; cholesterol; and polyethylene glycol (PEG)-lipid. It will be appreciated that the vehicle of medicaments according to the invention should be one which is well-tolerated by the subject to whom it is given.

5

Medicaments comprising the immunogenic composition of the invention may be used in a number of ways. For instance, oral administration may be required, in which case the agents may be contained within a composition that may, for example, be ingested orally in the form of a tablet, capsule or liquid. Compositions comprising agents and
10 medicaments of the invention may be administered by inhalation (e.g. intranasally). Compositions may also be formulated for topical use. For instance, creams or ointments may be applied to the skin.

The immunogenic composition of the invention may also be incorporated within a
15 slow- or delayed-release device. Such devices may, for example, be inserted on or under the skin, and the medicament may be released over weeks or even months. The device may be located at least adjacent the treatment site. Such devices may be particularly advantageous when long-term treatment with the immunogenic composition is required and which would normally require frequent administration (e.g. at least daily
20 injection).

In a preferred embodiment, however, medicaments according to the invention may be administered to a subject by injection into the blood stream, muscle, skin or directly into a site requiring treatment. Injections may be intravenous (bolus or infusion) or
25 subcutaneous (bolus or infusion), or intradermal (bolus or infusion), or intramuscular (bolus or infusion).

It will be appreciated that the amount of immunogenic composition that is required is determined by its biological activity and bioavailability, which in turn depends on the
30 mode of administration, the physiochemical properties of the immunogenic composition and whether it is being used as a monotherapy or in a combined therapy. The frequency of administration will also be influenced by the half-life of the active agent within the subject being treated. Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular the
35 immunogenic composition in use, the strength of the composition, the mode of administration, and the type and advancement of the viral infection. Additional factors

- 30 -

depending on the particular subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

Generally, a daily dose of between 0.001µg/kg of body weight and 100mg/kg of body weight of the immunogenic composition of the invention may be used for the immunisation, depending upon the agent used. More preferably, the daily dose of agent is between 1µg/kg of body weight and 100mg/kg of body weight, more preferably between 10µg/kg and 10mg/kg body weight, and most preferably between approximately 100µg/kg and 10mg/kg body weight.

Daily doses may be given as a single administration (e.g. a single daily injection or inhalation of a nasal spray). Alternatively, the immunogenic composition may require administration twice or more times during a day. As an example, the immunogenic composition may be administered as an initial primer and a subsequent boost(s), or two boosts administered at between a week or monthly intervals. Preferably, the immunogenic composition may be administered as an initial primer and a subsequent boost, administered between two to six weeks apart. Preferably, subsequent boosts may then be administered yearly to susceptible patients, such as those with weakened immune systems. Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials, etc.), may be used to form specific formulations of the immunogenic composition according to the invention and precise therapeutic regimes (such as daily doses of the agents and the frequency of administration).

A “subject” may be a vertebrate, mammal, or domestic animal. Hence, compositions and medicaments according to the invention may be used to treat any mammal, for example livestock (e.g. a horse), pets, or may be used in other veterinary applications. Most preferably, however, the subject is a human being.

A “therapeutically effective amount” of the immunogenic composition is any amount which, when administered to a subject, is the amount of the aforementioned that is needed to ameliorate, prevent or treat any given disease, preferably prophylactically.

For example, the immunogenic composition of the invention may be used from about 0.001 µg to about 1 mg, and preferably from about 0.001 µg to about 500 µg. It is

preferred that the amount of the immunogenic composition is an amount from about 0.01 µg to about 250 µg, and most preferably from about 0.1 µg to about 100 µg. Preferably, the immunogenic composition according to the invention is administered at a dose of 1-50 µg.

5

The immunogenic compositions of the invention may further comprise a pharmaceutically acceptable vehicle. A “pharmaceutically acceptable vehicle” as referred to herein, is any known compound or combination of known compounds that are known to those skilled in the art to be useful in formulating pharmaceutical compositions.

10

In one embodiment, the pharmaceutically acceptable vehicle may be a solid, and the composition may be in the form of a powder or tablet. A solid pharmaceutically acceptable vehicle may include one or more substances which may also act as
15 flavouring agents, lubricants, solubilisers, suspending agents, dyes, fillers, glidants, compression aids, inert binders, sweeteners, preservatives, dyes, coatings, or tablet-disintegrating agents. The vehicle may also be an encapsulating material. In powders, the vehicle is a finely divided solid that is in admixture with the finely divided active agents according to the invention. In tablets, the active agent (e.g. immunogenic
20 composition according to the invention) may be mixed with a vehicle having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active agents. Suitable solid vehicles include, for example calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidone,
25 low melting waxes and ion exchange resins. In another embodiment, the pharmaceutical vehicle may be a gel and the composition may be in the form of a cream or the like.

However, the pharmaceutical vehicle may be a liquid, and the pharmaceutical
30 composition is in the form of a solution. Liquid vehicles are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The immunogenic composition according to the invention may be dissolved or suspended in a pharmaceutically acceptable liquid vehicle such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid vehicle can
35 contain other suitable pharmaceutical additives such as solubilisers, emulsifiers, buffers, preservatives, sweeteners, flavouring agents, suspending agents, thickening

- 32 -

agents, colours, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid vehicles for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and
5 polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the vehicle can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid vehicles are useful in sterile liquid form compositions for parenteral administration. The liquid vehicle for pressurized compositions can be a halogenated hydrocarbon or other pharmaceutically
10 acceptable propellant.

Liquid pharmaceutical compositions, which are sterile solutions or suspensions, can be utilized by, for example, subcutaneous, intradermal, intrathecal, epidural, intraperitoneal, intravenous and particularly intramuscular injection. The nucleic acid
15 sequence, or expression cassette of the invention may be prepared as a sterile solid composition that may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium.

The immunogenic composition of the invention may be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents (for
20 example, enough saline or glucose to make the solution isotonic), bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like. The immunogenic composition according to the invention can also be administered orally either in liquid or solid
25 composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

30 It will be appreciated that the invention extends to any nucleic acid or peptide or variant, derivative or analogue thereof, which comprises substantially the amino acid or nucleic acid sequences of any of the sequences referred to herein, including variants or fragments thereof. The terms "substantially the amino acid/nucleotide/peptide
sequence", "variant" and "fragment", can be a sequence that has at least 40% sequence
35 identity with the amino acid/nucleotide/peptide sequences of any one of the sequences

referred to herein, for example 40% identity with the sequence identified as SEQ ID Nos: 1-27 and so on.

Amino acid/polynucleotide/polypeptide sequences with a sequence identity which is
5 greater than 65%, more preferably greater than 70%, even more preferably greater than 75%, and still more preferably greater than 80% sequence identity to any of the sequences referred to are also envisaged. Preferably, the amino acid/polynucleotide/polypeptide sequence has at least 85% identity with any of the sequences referred to, more preferably at least 90% identity, even more preferably at
10 least 92% identity, even more preferably at least 95% identity, even more preferably at least 97% identity, even more preferably at least 98% identity and, most preferably at least 99% identity with any of the sequences referred to herein.

The skilled technician will appreciate how to calculate the percentage identity between
15 two amino acid/polynucleotide/polypeptide sequences. In order to calculate the percentage identity between two amino acid/polynucleotide/polypeptide sequences, an alignment of the two sequences must first be prepared, followed by calculation of the sequence identity value. The percentage identity for two sequences may take different values depending on:- (i) the method used to align the sequences, for example,
20 ClustalW, BLAST, FASTA, Smith-Waterman (implemented in different programs), or structural alignment from 3D comparison; and (ii) the parameters used by the alignment method, for example, local vs global alignment, the pair-score matrix used (e.g. BLOSUM62, PAM250, Gonnet etc.), and gap-penalty, e.g. functional form and constants.

25 Having made the alignment, there are many different ways of calculating percentage identity between the two sequences. For example, one may divide the number of identities by: (i) the length of shortest sequence; (ii) the length of alignment; (iii) the mean length of sequence; (iv) the number of non-gap positions; or (v) the number of
30 equivalenced positions excluding overhangs. Furthermore, it will be appreciated that percentage identity is also strongly length dependent. Therefore, the shorter a pair of sequences is, the higher the sequence identity one may expect to occur by chance.

Hence, it will be appreciated that the accurate alignment of protein or DNA sequences
35 is a complex process. The popular multiple alignment program ClustalW (Thompson *et al.*, 1994, Nucleic Acids Research, 22, 4673-4680; Thompson *et al.*, 1997, Nucleic Acids

- 34 -

Research, 24, 4876-4882) is a preferred way for generating multiple alignments of proteins or DNA in accordance with the invention. Suitable parameters for ClustalW may be as follows: For DNA alignments: Gap Open Penalty = 15.0, Gap Extension Penalty = 6.66, and Matrix = Identity. For protein alignments: Gap Open Penalty = 10.0, Gap Extension Penalty = 0.2, and Matrix = Gonnet. For DNA and Protein alignments: ENDGAP = -1, and GAPDIST = 4. Those skilled in the art will be aware that it may be necessary to vary these and other parameters for optimal sequence alignment.

Preferably, calculation of percentage identities between two amino acid/polynucleotide/polypeptide sequences may then be calculated from such an alignment as $(N/T) \times 100$, where N is the number of positions at which the sequences share an identical residue, and T is the total number of positions compared including gaps and either including or excluding overhangs. Preferably, overhangs are included in the calculation. Hence, a most preferred method for calculating percentage identity between two sequences comprises (i) preparing a sequence alignment using the ClustalW program using a suitable set of parameters, for example, as set out above; and (ii) inserting the values of N and T into the following formula:- Sequence Identity = $(N/T) \times 100$.

Alternative methods for identifying similar sequences will be known to those skilled in the art. For example, a substantially similar nucleotide sequence will be encoded by a sequence which hybridizes to DNA sequences or their complements under stringent conditions. By stringent conditions, the inventors mean the nucleotide hybridises to filter-bound DNA or RNA in 3x sodium chloride/sodium citrate (SSC) at approximately 45°C followed by at least one wash in 0.2x SSC/0.1% SDS at approximately 20-65°C. Alternatively, a substantially similar polypeptide may differ by at least 1, but less than 5, 10, 20, 50 or 100 amino acids from the sequences shown in, for example, in those of SEQ ID Nos: 1 to 27 that are amino acid sequences.

Due to the degeneracy of the genetic code, it is clear that any nucleic acid sequence described herein could be varied or changed without substantially affecting the sequence of the protein encoded thereby, to provide a functional variant thereof. Suitable nucleotide variants are those having a sequence altered by the substitution of different codons that encode the same amino acid within the sequence, thus producing a silent (synonymous) change. Other suitable variants are those having homologous nucleotide sequences but comprising all, or portions of, sequence, which are altered by

- 35 -

the substitution of different codons that encode an amino acid with a side chain of similar biophysical properties to the amino acid it substitutes, to produce a conservative change. For example, small non-polar, hydrophobic amino acids include glycine, alanine, leucine, isoleucine, valine, proline, and methionine. Large non-polar, hydrophobic amino acids include phenylalanine, tryptophan and tyrosine. The polar neutral amino acids include serine, threonine, cysteine, asparagine and glutamine. The positively charged (basic) amino acids include lysine, arginine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. It will therefore be appreciated which amino acids may be replaced with an amino acid having similar biophysical properties, and the skilled technician will know the nucleotide sequences encoding these amino acids.

All of the features described herein (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined with any of the above aspects in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

For a better understanding of the invention, and to show how embodiments of the same may be carried into effect, reference will now be made, by way of example, to the accompanying Figures, in which:-

Figure 1 shows a heatmap visualising pairwise identity scores between 150 rhinovirus strain VPo protein sequences calculated using the MUSCLE algorithm after hierarchical clustering. Pairwise identity scores above (>59.9%, blue) or below (<59.9%, red) the thresholds predicted to be required for cross-immunization are shown.

Figure 2 shows circular tree clustering 150 rhinovirus strain VPo protein sequences into 3 clusters. Of the sequences analysed, cluster 1 (red) contains all RV-A species VPo sequences, cluster 2 (black) contains all RV-B species VPo sequences and cluster 3 (green) contains all RV-C species VPo sequences.

Figure 3 is a set of histograms representing the number of IFN- γ producing splenocytes from mice immunised with RV-A16 VPo protein after stimulation with RV-A16 VPo (A) or the indicated RV-A (B) or RV-B/C (C) species peptide pools. Mice were sacrificed 35-49 days after first immunisation, splenocytes harvested, stimulated as

- 36 -

indicated and IFN- γ producing splenocytes (/10⁶ splenocytes) enumerated by ELISPOT. (A) & (B) n=24 mice, data pooled from 3 independent experiments; (C) n=5 mice. Data is presented as geomean $\pm$ 95% confidence intervals. Significant differences were compared to irrelevant peptide using a linear mixed effects model ***: p<0.0001; n.s.: non-significant.

Figure 4 is a set of histograms representing the number of IFN- γ producing splenocytes from mice immunised with RV-Bo6 VPO protein after stimulation with RV-Bo6 VPO (A) or the indicated RV-B (B) or RV-A/C species peptide pools (C). Mice were sacrificed 35-49 days after first immunisation, splenocytes harvested, stimulated as indicated and IFN- γ producing splenocytes (/10⁶ splenocytes) enumerated by ELISPOT. (A) & (B) n=24 mice, data pooled from 3 independent experiments; (C) n=7 mice. Data is presented as geomean $\pm$ 95% confidence intervals. Significant differences were compared to irrelevant peptide using a linear mixed effects model. ***: p<0.0001; **: p<0.001; *p<0.01; n.s.: non-significant.

Figure 5 is a set of histograms representing the number of IFN- γ producing splenocytes from mice immunised with RV-Co7 VPO protein after stimulation with RV-Co7 VPO (A) or the indicated RV-C (B) or RV-A/B species (C) peptide pools. Mice were sacrificed 35-49 days after first immunisation, splenocytes harvested, stimulated as indicated and IFN- γ producing splenocytes (/10⁶ splenocytes) enumerated by ELISPOT. n=7 mice; data pooled from 2 independent experiments. Data is presented as geomean $\pm$ 95% confidence intervals. Significant differences were compared to irrelevant peptide using a linear mixed effects model. ***: p<0.0001; **: p<0.01; *p<0.05; n.s.: non-significant.

Figure 6 is a set of dot plot showing the median fragment identity/similarity scores for each RV-C strain VPO sequence across all other RV-C VPO strains plotted against the median sequence identity/similarity score for each strain against RV-Co7 alone. (A) All C strains plotted as a single cluster. (B) C strains split into two clusters.

Figure 7 is a set of histograms representing the number of IFN- γ producing splenocytes from mice immunised with RV-Co1 VPO protein after stimulation with RV-Co1 VPO (A) or the indicated RV-C (B) or RV-A/B species (C) peptide pools. Mice were sacrificed 35-49 days after first immunisation, splenocytes harvested, stimulated as indicated and IFN- γ producing splenocytes (/10⁶ splenocytes) enumerated by

ELISPOT. n=7 mice; data pooled from 2 independent experiments. Data is presented as geomean $\pm$ 95% confidence intervals. Significant differences were compared to irrelevant peptide using a linear mixed effects model. ***: $p < 0.0001$; **: $p < 0.01$; * $p < 0.05$; n.s.: non-significant.

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Figure 8 is a set of histograms representing the number of IFN- γ producing splenocytes from mice immunised with RV-C19 VPo protein after stimulation with RV-19 VPo (A) or the indicated RV-C (B) or RV-A/B (C) species peptide pools. Mice were sacrificed 35-49 days after first immunisation, splenocytes harvested, stimulated as indicated and IFN- γ producing splenocytes ($/10^6$ splenocytes) enumerated by ELISPOT. N=12 mice; data pooled from 2 independent experiments. Data is presented as geomean $\pm$ 95% confidence intervals. Significant differences were compared to irrelevant peptide using a linear mixed effects model. ***: $p < 0.0001$; **: $p < 0.001$; * $p < 0.01$; n.s.: non-significant.

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Figure 9 is a set of histograms representing the number of IFN- γ producing splenocytes from mice immunised with RV-C24 VPo protein after stimulation with RV-C24 VPo (A) or the indicated RV-C (B) or RV-A/B (C) species peptide pools. Mice were sacrificed 35-49 days after first immunisation, splenocytes harvested, stimulated as indicated and IFN- γ producing splenocytes ($/10^6$ splenocytes) enumerated by ELISPOT. (A) & (B) n=26 mice, data pooled from 2 independent experiments; (C) n=7 mice. Data is presented as geomean $\pm$ 95% confidence intervals. Significant differences were compared to irrelevant peptide using a linear mixed effects model. ***: $p < 0.0001$.

Figure 10 is a line graph representing the titre of anti-RV VPo antibodies from mice immunised twice with either RV-Bo6, RV-Co1, RV-Co7, RV-C19 or RV-C24 VPo proteins or controls (PBS or vaccine adjuvant alone). Serum was collected 42 days after first immunisation and the titres of immunogen-specific IgG2a were quantified by ELISA. Each curve represents the titre of the anti-RV VPo IgG2a specific to VPo protein the mice were immunised with (n=7 mice/immunogen, mean $\pm$ SEM). Sera from control immunised mice (adjuvant alone or PBS, n=7 per group) were pooled and the respective pooled sera tested in each VPo-specific ELISA (mean $\pm$ SEM of responses across VPo antigens).

Figure 11 is a set of histograms that show the results from ELISPOT experiments to analyse the cellular immunity evoked in cynomolgus monkeys by immunisation with

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- 38 -

rRV-C24 VPo protein (Figure 11B) and adjuvant only (control; Figure 11A) against other strains of RV. Bars represent mean activity/ 2×10^5 PBMCs from 3 technical replicates $\pm$ SEM.

5 **Figure 12** shows IFN- γ secretion (Figure 12A) and IL-4 secretion (Figure 12B) in cynomolgus monkeys immunised with rRV-C24 VPo protein or adjuvant only (control), in response to stimulation with peptide pools from C species of RV. Bars represent mean activity/ 2×10^5 PBMCs from 3 technical replicates $\pm$ SEM.

10 **Figure 13** is a histogram showing the concentration of RV-C24 VPo-binding IgG, as quantified by ELISA, in the plasma of cynomolgus monkeys at different timepoints after immunization with rRV-C24 VPo protein. Bars represent mean IgG concentration, and dots represent individual animal responses. Arrows indicate when animals were immunized.

15

Figure 14 is a Western blot of HEK293 lysates made at different timepoints after transfection with RV VPo mRNA. The marker lane is designed M, with bands at 38 and 28 kDa labelled. The predicted molecular weight of RV VPo protein is approximately 36 kDa. Figure 14A is a Western blot of lysates from HEK293 cells transfected with RV-A16 VPo mRNA made at 24, 48, 72 or 96 hours after transfection. A positive control of recombinant RV-A16 VPo protein was loaded onto the gel (labelled rRV-A16 VPo). Detection of RV-A16 VPo protein was performed using a monoclonal anti-RV-A16 VPo antibody. Figure 14B is a Western blot of lysates from HEK293 cells transfected with RV-Bo6 or RV-C24 VPo mRNA made at 24, 48, 72 or 96 hours after transfection. Detection of RV-Bo6 and C24 VPo proteins was performed using antisera from mice immunized with either recombinant RV-Bo6 or recombinant RV-C24 VPo protein, respectively.

25 **Figure 15** is a histogram that shows the results from ELISPOT experiments to analyse the cellular immunity in mice evoked by immunisation with RV-C24 VPo mRNA against other strains of RV. Bars represent mean activity/ 5×10^5 splenocytes $\pm$ SEM from 6 animals. * $p < 0.05$, ANOVA.

30 **Figure 16** is a histogram showing the concentration of RV-C24 VPo-binding IgG, as quantified by ELISA, in the serum of mice at different timepoints after immunization

with RV-C24 VPo mRNA. Bars indicate mean IgG concentration $\pm$ SEM. Arrows indicate when animals were immunized.

Figure 17 is a histogram that show the results from ELISPOT experiments to analyse the cellular immunity evoked by immunization of cynomolgus monkeys with an mRNA concatemer consisting of RV-C24/A16/Bo6 VPo mRNAs in sequence separated by a 2A peptide-encoding sequence. IFN- γ (Figure 17A) or IL-4 (Figure 17B) secretion was quantified by ELISPOT. Bars represent mean activity/ 2×10^5 PBMCs from 3 technical replicates $\pm$ SEM.

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Figure 18 is a histogram showing the concentration of RV-A16 (Figure 18A), RV-Bo6 (Figure 18B) and RV-C24 (Figure 18C) VPo-binding IgG, as quantified by ELISA, in the plasma at different timepoints after immunization of a cynomolgus monkey with an mRNA concatemer consisting of RV-C24/A16/Bo6 VPo mRNAs in sequence separated by a 2A peptide-encoding sequence. Arrows indicate when the animal was immunized. Bars indicate the IgG concentration detected in a single animal. Each graph was generated using data from the same animal.

Figure 19 is a histogram that shows the concentration of RV-A01 RNA in the lungs of control and RV-A16 VPo mRNA immunized mice, as determined by RT-qPCR, 1, 6 and 14 days after infection with RV-A01. Bars represent mean RV-A01 RNA $\pm$ SEM from 8 animals. * $p < 0.05$, ANOVA.

Figure 20 is a histogram that shows the results from ELISPOT experiments to analyse how cellular immunity in mice formed by immunisation with RV-A16 VPo mRNA responds to subsequent infection with heterotypic RV-A01 in splenocytes harvested 14 days after infection with RV-A1. Bars represent mean spot forming units/ 1×10^6 splenocytes $\pm$ SEM from 8 animals. * $p < 0.05$, *** $p < 0.001$, ANOVA.

Figure 21 is a histogram that shows the results from ELISPOT experiments to analyse cellular immunity from control mice whose spleens were also harvested 14 days after infection with RV-A01. Bars represent mean spot forming units/ 1×10^6 splenocytes $\pm$ SEM from 8 animals.

Figure 22 is a histogram that shows the mean numbers of CD62⁻ CD44⁺ effector CD4⁺ (Figure 22A) and CD8⁺ (Figure 22B) T cells, respectively, in the lungs of RV-A16 VPo

- 40 -

mRNA immunized and control mice after infection with RV-A1. Bars represent mean cell numbers +/- SEM from 8 animals. **p<0.01, ***p<0.001, ANOVA.

Figure 23 is a set of correlation plots where the frequency of Th1 cells is plotted
5 against the frequency of Th2 cells for each mouse infected with RV-A1 after
immunization with RV-A16 VPo mRNA (Figure 23B) or control (Figure 23A).

Figure 24 is a set of histograms showing the number of CD4⁺ (Figure 24A) and CD8⁺
(Figure 24B) T_{RM} cells in the BAL of control and RV-A16 VPo mRNA immunized mice
10 at different post-RV-A1 infection timepoints. Bars represent mean cell numbers or
mean cell frequency +/- SEM from 8 animals. ****p<0.0001, ANOVA.

Figure 25 is a set of histograms showing the frequency of CD4⁺ (Figure 25A) and CD8⁺
(Figure 25B) T_{RM} cells in the BAL of control and RV-A16 VPo mRNA immunized mice
15 at different post-RV-A1 infection timepoints. Bars represent mean cell numbers or
mean cell frequency +/- SEM from 8 animals. *p<0.05, **p<0.01, ***p<0.001,
****p<0.0001, ANOVA.

Figure 26 is a set of histograms showing the number of CD4⁺ (Figure 26A) and CD8⁺
20 (Figure 26B) T_{RM} cells in the lungs of control and RV-A16 VPo mRNA immunized mice
at different post-RV-A1 infection timepoints. Bars represent mean cell numbers or
mean cell frequency +/- SEM from 8 animals. *p<0.05, **p<0.01, ***p<0.001,
ANOVA.

Figure 27 is a set of histograms showing the frequency of CD4⁺ (Figure 27A) and CD8⁺
(Figure 27B) T_{RM} cells in the lungs of control and RV-A16 VPo mRNA immunized mice
25 at different post-RV-A1 infection timepoints. Bars represent mean cell numbers or
mean cell frequency +/- SEM from 8 animals. ****p<0.0001, ANOVA.

30 Materials and Methods

Bioinformatical analysis to identify centroid RV VPo immunogens

RV VPo sequences

RV VPo protein sequences were extracted manually from the NCBI protein database
35 using a mixture of blast and annotation search. The results were refined by comparing

to the strains annotated in Taxonomy Browser (NCBI). 150 RV strain VPo protein sequences were used in the analysis: 78 RV-A, 25 RV-B and 47 RV-C sequences.

Alignments, sequence identity and similarity scores

- 5 Multiple alignment of the 150 RV VPo sequences was performed using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm. From the alignment, identity and similarity matrices were extracted. The identity matrix was used to calculate the percentage of identical amino acids per position between all sequences pairwise. The similarity matrix was used to calculate the percentage of amino acids with
10 similar physicochemical properties per position between all sequences pairwise.

Hierarchical clustering

- The 150 RV strain VPo sequences were clustered based on the calculated identity scores (transformed in distances, as required by the method) using non-supervised
15 hierarchical clustering. The tree was then split into three clusters (Figure 1). From each cluster, a centroid RV VPo strain sequence (i.e. central/typical strain or medoid) was identified. Each centroid VPo sequence was defined as the VPo sequence within that cluster that was predicted to generate an immune response against the maximum percentage of other strains within that cluster. Centroid identification was performed
20 using a range of identify threshold as defined below.

Fragment analysis

- The conservation among RV strain VPo protein sequences was also analysed at the epitope level, by splitting each strain VPo sequence into 12-mers with a one amino acid
25 overlap. Fragment identity was estimated by calculating the percentage of conserved 12-mer fragments between VPo sequence pairs (i.e. “conserved” is defined as 100% identity between two fragments). Fragment similarity was estimated by calculating the percentage of similar 12-mer fragments; similar being defined as 80% of the amino acids in the fragments being conserved or evolutionarily “interchangeable” as per
30 BLOSUM62 scoring).

RV VPo protein production and purification

Table 1 - List of plasmids used to produce the different RV VPo immunogens.

Plasmid ID	RV strain VPo
pETSumoRV RVA16 VPo	RV-A16
pETSumoRV-B-DGW2, VP_D	RV-Bo6
pETSumoRV-C1	RV-Co1

pETSumoRV-C-DGV2, VP_E	RV-Co7
pETSumoRV-C19RV	RV-C19
RV pETSumoRV-C24	RV-C24

The same cloning strategy has been applied for all recombinant proteins. Briefly, each respective nucleotide sequence was optimized for *E. coli* expression and the synthetic gene was cloned in frame with the SUMO sequence in the T/A cloning site of the pET-SUMO vector and then expressed using the pET-SUMO expression system (Invitrogen). Genes were synthesised and plasmids constructed at Crelux GmbH or Charles River Laboratories.

Escherichia coli (T7 Express, NEB) were transformed with either of the plasmids listed above. Expression was carried out on 1-2 litre scale in shake flasks (Ultra Yield, Thomson) using 2YT media. The cultures were grown to an OD_{600nm} of 0.8 at 37°C and protein production was induced by the addition of 1mM IPTG with the temperature reduced to 18°C prior to induction. The cells were harvested 16 hours post induction by centrifugation for 10 min at 4,500 g and pellets either stored at -80 °C or processed immediately.

Progression of the purification was monitored by SDS-PAGE after each purification step. Purification was performed at 4°C using chilled buffers (buffer components are listed in Table 2 below). Pellets were re-suspended in Buffer A (10 mL/ 1 g of cells) and broken using a cell disrupter (Constant Systems) at 25 kpsi. Lysates were cleared by centrifugation for 30 min at 38,000 g. The resulting inclusion body pellets were washed in the same buffer a further two times and solubilised overnight at 4°C in Buffer B (10 mL/ 1 g of cells lysed) by stirring. This solution was then clarified by centrifugation for 30 minutes at 38,000 xg and loaded onto two consecutively connected 5 mL HisTrap HP column (Cytiva™) equilibrated in Buffer B at 1 mL/min using an Aktaexpress system. The columns were washed with Buffer B until the baseline was stable which was followed by a 20 CV wash with Buffer C and re-equilibration into Buffer B by a further 20 CV wash. After that a shallow gradient over 30 CV was applied into Buffer D after which the protein was eluted using Buffer E followed by immediate buffer exchange into buffer F using a HiPrep™ 26/10 desalting column (Cytiva™).

To remove the His-SUMO-tag ~ 0.4U of Sumo protease (Lifesensors Inc) per 1 mg of VPo protein were added to the eluted protein and incubated overnight at 4°C.

The cleaved protein was separated from the uncleaved material and protease by secondary nickel chromatography in gravity flow using HisSelect® Affinity Gel (Cytiva™) equilibrated in Buffer F.

- 5 The flow through was collected, concentrated to ~ 2 mL and loaded onto a Superdex S200 gel filtration column (Cytiva™) equilibrated in Buffer F run at 1 mL/min. Fractions containing VPO protein were pooled for final overnight dialysis into Buffer G.

- 10 The dialysed samples were then concentrated between to 0.3-0.6 mg/ml before tested for their Endotoxin levels using the ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript®) following the manufacturer's instructions. Samples that had lower levels than 5 EU/ mg of protein were aliquoted and stored at -80°C.

Table 2 – Constituents of buffers used for RV VPO protein purification

Buffer	Purification step	Components
A	Cell lysis	50 mM Tris pH 8.0, 150 mM NaCl
B	Solubilisation	20 mM Tris pH 8.0, 150 mM NaCl, 8 M Urea, 10 mM imidazole
C	Triton X-114 wash	20 mM Tris pH 8.0, 150 mM NaCl, 8 M Urea, 10 mM imidazole, 0.2% Triton X-114
D	2 M Urea buffer for gradient	20 mM Tris pH 8.0, 150 mM NaCl, 2 M Urea, 10 mM imidazole
E	Elution buffer	20 mM Tris pH 8.0, 150 mM NaCl, 2 M Urea, 400 mM imidazole
F	Desalt/Sizing buffer	20 mM Tris pH 8.0, 150 mM NaCl, 2 M Urea
G	Dialysis/ Refolding buffer	20 mM Tris pH 8.0, 150 mM NaCl, 0.5 M arginine

15

Mouse immunisation

8–10-week-old C57BL/6 mice were immunised by subcutaneous (SC) route on day 0 and day 21.

20

Each mouse was given 10 µg of RV VPO protein (either RV-A16, RV-Bo6, RV-Co1, RV-Co7, RV-C19 or RV-C24) in a total volume of 100 µl with IFA/CpG adjuvant (10 µg CPG

- 44 -

1826 (Invivogen, Toulouse France) + 100 µl Incomplete Freund's Adjuvant (IFA Sigma-Aldrich Gillingham United Kingdom).

Protein buffer (Tris 20 mM, NaCl 150 mM, Arginine 0.5 M pH 8.0) in the presence or
5 absence of IFA/CpG adjuvant was used as a negative control and administered in control groups of mice according to the same procedure.

Sample Processing

Blood and spleens were collected on day 42. Blood was collected in Vacutainer tubes
10 (BD Vacutainer SST II Nus plastic serum tube (BD Biosciences, Le Pont-De-Claix, France), kept overnight at 4°C and centrifuged for 20 minutes at 1660 g to separate serum from cells. Sera were stored at -20°C.

Spleens were rapidly collected after sacrifice under sterile conditions.

15

Cytokine ELISPOTs

Spleens were homogenized manually with a syringe plunger through a cell strainer (BD Biosciences, San Jose, Calif.) and treated with Red Blood Cell Lysing Buffer HybriMax (Sigma-Aldrich Gillingham, United Kingdom) to lyse red cells. Cells were washed two
20 times with RPMI 1640 medium with HEPES (Gibco, Paisley, UK), supplemented with 2% of decompemented foetal calf serum (FCS) (HYCLONE Hyclone, Logan, Utah), 50 µM of 2-mercaptoethanol (Gibco), 2 mM of L-glutamine (Gibco) and 100 units/mL of Penicillin-Streptomycin (Gibco). Cells were counted on a Multisizer and resuspended in complete medium with RPMI 1640 medium (Gibco), supplemented with 10% of
25 decompemented FCS (HYCLONE), 50 µM of 2-mercaptoethanol (Gibco), 2 mM of L-glutamine (Gibco) and 100 units/mL of Penicillin-Streptomycin (Gibco). 2×10^5 splenocytes per well were seeded in 96 well plates (source) and stimulated with pools of peptides corresponding to the different RV-Bo6, RV-Co1, RV-Co7, RV-C19 or RV-C24 full-length VPO antigens plus murine IL-2 at 20 U/ml. In addition, to assess cross-
30 reactivity the splenocytes were also stimulated with peptide pools corresponding to full length VPO from a range of RV strains from each RV species, as indicated in each of the figures. Each peptide pool consisted of 80 15-mer peptides covering the entirety of the VPO protein, overlapping by 11 amino acids. Peptide pools were synthesised by Mimotopes and used at a final concentration of 1 µg/ml. Phorbol 12-myristate 13-
35 acetate (PMA; Invivogen Toulouse France) plus ionomycin (Stemcell, Cambridge United Kingdom) was used as a positive stimulation control. An irrelevant peptide pool

- 45 -

(PepMix™ Human (HLA class I Ig-like C1 type domain), sourced from JPT (Berlin Germany), 15-mers with 11 amino acid overlap) was used as a negative stimulation control. Plates had been previously coated overnight at 4°C with rat anti-mouse IFN- γ antibody (BD Pharmingen, San Diego, Calif.) at 1 μ g per well in sterile PBS and blocked
5 for one hour at 37°C with 10% FCS complete RPMI 1640 medium (Gibco). Stimulation of splenocytes was performed for 18 hours at 37°C with 5% CO₂.

After splenocyte stimulation, plates were washed three times with PBS and then three times with PBS -Tween 0.05%. Biotinylated anti-IFN- γ (BD Biosciences, Le Pont-De-
10 Claix, France), 100 μ L per well, was added at 5 mg/ml in PBS-Tween 0.05% and the plates incubated at 20°C in the dark. Plates were then washed 3 times with PBS-Tween 0.05% and incubated with streptavidin-horseradish peroxidase (Southern Biotech) in PBS-Tween 0.05% for 1 hour at 20°C in the dark.

15 The plates were next washed three times with PBS-Tween 0.05%, followed by three times with PBS. 100 μ L per well of substrate solution (3-amino-9-ethylcarbazole, AE, Sigma-Aldrich) was added and the plates incubated for 15 min at 20°C in the dark. The reaction was stopped by adding approximately 150 μ L per well of distilled water. Substrate solution was prepared by mixing: 9 ml distilled water, 1 ml acetate buffer,
20 0.250 ml AE and 5 μ L H₂O₂. The solution was filtered through a 0.22 μ m filter (Sigma-Aldrich). Each spot, corresponding to an IFN- γ secreting T cell was enumerated with an automatic ELISPOT reader (AID, Strassberg Germany). Results were expressed as number of IFN- γ spots per 10⁶ splenocytes.

25 The ELISPOT data for each immunogen were analysed separately on the log₁₀ scale using a linear mixed model containing fixed effects for study group and treatment group and a random effect adjusting for individual mice. Results for each treatment group were reported on the back transformed (anti-logged) scale using least square geometric means, ratios of least square geometric means, 95% confidence intervals and
30 p-values for individual comparisons. Statistical significance was reported at 5% significance level (i.e. p<0.05). No multiple comparison adjustment was performed as all comparisons were defined a priori and were against the control group (irrelevant peptide).

35 ELISA

- 46 -

Anti-RV VPo IgG2a (or IgG2c responses in 57Bl/6 mice) responses were measured by ELISA. Greiner 96-well microplates (Greiner, Gloucestershire, United Kingdom) were coated with 100 ng per well of RV-Bo6, C19 or C24 VPo in PBS buffer pH 7.4 (Sigma-Aldrich) overnight at 4°C. Non-specific sites were blocked with 150 µl per well of PBS-Tween (PBS pH 7.1, 0.05% Tween 20) plus 1% skimmed milk for one hour at 37°C. Sera were serially diluted 2-fold in the VPo coated plates from starting dilutions of 1:100 or 1:1000 in PBS-Tween 0.05%, milk 1%, incubated for 90 minutes and washed three times with PBS-Tween.

RV VPo-specific IgG2a was detected by adding goat anti-mouse IgG2a conjugated to HRP (Southern Biotech, Birmingham, Ala.) diluted 1:4000 in PBS-Tween plus 1% skimmed milk to each well and incubating for 90 minutes at 37°C.

The plates were then washed three times with PBS-Tween and TetraMethylBenzidine (TMB, Tebu-bio laboratories, Le Perray-en-Yvelines, France) substrate solution added to each well at 100 µL per well. The plates were incubated for 10-30 minutes in the dark at room temperature. The colorimetric reaction was stopped by the addition of 100 µl/well of 1M HCl (VWR Prolabo Fontenay-sous-Bois, France). The plates were immediately read at 450 and 650 nm on a Versamax plate reader (Molecular Devices).

20

In vitro mRNA studies

Transfection of HEK cells with VPo mRNA & detection of expressed RV VPo proteins

HEK293 cells were cultured in DMEM medium supplemented with 10% FCS and 1% penicillin/streptomycin. For transfection, cells were plated in 24 well plates and transfected in duplicate at approximately 80% confluency. Cells were transfected with RV VPo mRNA with mRNA-Fect (RJH Biosciences) according to a pre-optimised protocol consisting of 4 µL per well of mRNA (Tri-Link), 60 µL of serum free DMEM medium and 4 µL of mRNA-lect. An untransfected control was included and consisted of mRNA-lect without any mRNA. Prior to transfection, each mRNA was diluted to 0.1 mg/mL in RNase free water (Promega) and mRNA-lect was used as a neat solution of 1 mg/mL according to the manufacturer's recommended protocol. Diluted mRNA (4 µL) was added to 60 µL DMEM medium and vortexed for 3sec. Undiluted mRNA-lect (4 µL per transfection), was then added and the RNA mixtures vortexed for 10sec and then left at room temperature for 30min to complex. After incubation, the complexes (68 µL in total) were added dropwise directly to each well of a 24 well plate in duplicate, in

- 47 -

which the medium volume was 0.5 mL. Cells were then incubated for a set period of time (24-96h) prior to harvest. For sample harvests, the medium was removed, monolayers washed in 0.5 mL PBS pH 7.4, and 100 µL of 2X SDS sample buffer (Invitrogen) added over the two duplicate wells. The cells were lysed by both pipetting and scraping, duplicates were then pooled, producing 1 sample and samples were stored at -80°C. Protein expression was visualised using SDS-polyacrylamide gel electrophoresis (SDS-PAGE, Invitrogen) followed by transfer of proteins onto nitrocellulose (Invitrogen), and western blotting. Western blotting consisted of probing each membrane with pooled VPO specific mouse anti-sera (anti-A16, Bo6 or C24 VPO) diluted 1/200 in blocking buffer (5% skim milk in PBS) overnight at 4°C. In certain experiments, the membranes were probed with an anti-RV-A16 monoclonal antibody (Antibodies Online, Cat No. ABIN1000236) diluted 1/500 in blocking buffer overnight. Following incubation, membranes were washed 3X in 30-50 mL washing buffer (PBS pH 7.4 with 0.1% Tween 20) for 5min with shaking. After washing, an anti-mouse H+L chain IgG-HRP secondary antibody (Invitrogen) was added diluted 1/5,000 in blocking buffer. The secondary antibody was incubated for 1h with shaking, the secondary removed, and 30-50mL of washing buffer added for 30min, followed by 2X washes of 30-50mL washing buffer for 5 min each. After washing, 2 mL of ECL Plus substrate (Thermo Scientific) was added for 2min and the membranes subjected to image acquisition using a Peqlab imager according to the manufacturer's recommended protocol for chemiluminescence.

Cynomolgus monkey mRNA vaccine studies

All studies were performed under local laws and regulations and were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

mRNA vaccine preparation

On the day of use, *in vivo*-jetRNA® transfection reagent and mRNA buffer (both Polyplus, France) were equilibrated to room temperature. mRNA was diluted in mRNA buffer to 50 µg/mL. A volume of *in vivo*-jetRNA® transfection reagent equal to the volume of mRNA added to the mRNA buffer was added and the resulting solution was incubated for 15 minutes at room temperature prior to use. For control solutions, the mRNA was replaced with an equal volume of sterile water (Polyplus).

Cynomolgus monkey immunization

- 48 -

Each animal received 100 µg of mRNA in a total volume of 2 mL, or 2 mL transfection control reagent, dosed intramuscularly on Days 0 and 21 of the study.

Blood sampling and preparation of plasma and peripheral blood mononuclear cells

5 Blood was collected on Days 0, 14, 21, 28, 35 and 42 of the study from the saphenous or cephalic veins. The blood was incubated at room temperature for 30 minutes before being centrifuged and plasma and peripheral blood mononuclear cells (PBMCs) prepared by standard methods.

10 Plasma ELISA

96-well plates were coated with Goat Anti-Rhesus IgG(H+L)-UNLB (Southern Biotech, U.S.) diluted in PBS for the standard curve, or 25 µg/well RV VPO protein diluted in PBS for detection of antigen-specific IgG in monkey plasma samples. Plates were incubated overnight at 4°C. Plates were washed three times with PBS-Tween (PBS
15 containing 0.05% Tween 20 (Sigma-Aldrich)). Non-specific binding was blocked by incubation with PBS 5% milk (Sigma-Aldrich) for 2 hours at room temperature. Plates were washed three times with PBS-Tween. 100 µL standard or plasma sample were added per well, diluted in PBS-1% BSA and incubated for 2 hours at room temperature. An 11-point standard curve was generated using serial 2-fold dilutions of Rhesus
20 Monkey IgG-UNLB (Southern Biotech, U.S) starting from 400 ng/mL. Plates were washed three times with PBS-Tween. Bound antibody was detected with Peroxidase AffiniPure Goat Anti-Human IgG, Fcy fragment specific (Jackson ImmunoResearch, U.S.) diluted 1:5000 in PBS 1% BSA (Sigma-Aldrich), incubated for 1 hour at room temperature. Plates were washed three times with PBS-Tween, then developed using
25 100 µL TMB substrate per well incubated for approximately 10 minutes, followed by 100 µL 0.18M H₂SO₄ per well to stop the reaction. Plates were read immediately at 450nm using a SpectraMax plate reader (Molecular Devices).

PBMC ELISpot

30 PBMCs were stimulated with peptide pools as described above using 2x10⁵ cells per peptide pool stimulation. Each stimulation was performed in triplicate. IFN-γ or IL-4 ELISpot was performed using Monkey IFN-γ ELISpot PRO kit (ALP), strips (Mabtech-3421M-2AST-10) and Human IL-4 ELISpot PRO kit (ALP) (Mabtech-3410-2APW-10), according to the manufacturers instructions. The activity per 2x10⁵ PBMCs was
35 determined using an AID ELISpot machine (Model ELRo88IFL).

Mouse mRNA vaccine studies

mRNA Vaccine Preparation

On the day of use, *in vivo*-jetRNA® transfection reagent and mRNA buffer (both
5 Polyplus, France) were equilibrated to room temperature. mRNAs were diluted in
mRNA buffer to 200µg/mL. An equal volume of *in vivo*-jetRNA® transfection reagent
was added to the diluted mRNA, and the resulting solution was incubated for 15
minutes at room temperature prior to use. For control solutions, the mRNA was
replaced with an equal volume of sterile water (Polyplus).

10

Mouse Immunisation

Each mouse received 5µg RV mRNA vaccine in a total volume of 50 µL, or 50 µL
transfection control reagent, dosed intramuscularly on Days 0 and 21 of the study.

15 Mouse rhinovirus infection

Each mouse was dosed intranasally (i.n.) with 50µL rhinovirus preparation (grown as
described in Bartlett *et al*, 2008, Nat. Med.) or 50µL PBS (ThermoFisher Scientific,
UK), administered dropwise to both nares whilst under isoflurane anaesthesia.

20 Mouse sample harvesting and processing

Blood samples were obtained at timepoints prior to sacrifice via tail vein puncture and
collected using Na-heparinised capillary tubes (Hirschmann-Laborgeräte, Germany).
Terminal blood samples were collected from the jugular vein using Na-heparinised
capillary tubes. Whole blood samples were centrifuged to isolate serum from cells, and
25 serum was subsequently stored at -80 °C.

Following euthanasia, the trachea was cannulated and bronchoalveolar lavage (BAL)
was performed using three 0.5mL sterile PBS washes per mouse, pooled to give a total
volume of 1.5 mL BAL. Samples were centrifuged at 1500 RPM for 10 min at 4°C and
30 supernatants were stored at -80°C. Cells were re-suspended in 0.2% w/v NaCl to
haemolyse red blood cells, followed by an equal volume of 1.6% w/v NaCl.

Lymph nodes and spleens were harvested into R10 medium (RPMI 1640 medium
containing 10 % foetal calf serum (FCS) and 100 U/mL penicillin/streptomycin (P/S),
35 all ThermoFisher Scientific), then homogenised manually through 100µm filters

- 50 -

(Greiner Bio-One, UK). Red blood cells were lysed using ACK Lysing Buffer (Fisher Scientific, UK) and cells were then washed twice with R10 medium.

The superior right lung lobe and post caval lung lobe from each mouse were harvested
5 into RNeasy Protect (Qiagen, UK) and stored at -80°C. The remaining lung lobes were
collected into R10 medium then diced finely and incubated at 37°C for 30 minutes in
R10 medium supplemented with 0.14 Wünsch units/mL Liberase (Roche, UK) and 50
µg/mL DNase1 (Roche). Lung tissue was then manually homogenised through 100µm
10 filters (Greiner Bio-One) Red blood cells were lysed using ACK Lysing Buffer (Fisher
Scientific, UK) and cells were then washed twice with R10 medium.

Viability of single cell preparations was assessed using trypan blue (ThermoFisher Scientific) exclusion staining and total cells were enumerated using a haemocytometer (Neubauer, Germany).

15

Neutralisation assay using mouse serum

Mouse sera were serially diluted 1 in 2 or 1 in 3.14 and incubated with RV-A1 (5x10⁴ TCID₅₀ per mL) in an 8-point titration curve for 1h with shaking. The virus-serum complexes (50 µL per well) were then incubated with 150 µL per well of HeLa cells
20 (3x10⁵ cells per mL) in replicates of eight in flat bottomed 96 well plates. Cells with
virus, and cells only were used as controls. In some experiments, anti-RV-A1 guinea pig
sera (ATCC Cat No V-113-501-558) was used as a positive control for neutralisation
through serial 1 in 3.14 dilutions creating a 12-point titration curve. Plates were
incubated for 3 days and then supernatants were discarded, and cell monolayers
25 stained with 150 µL per well crystal violet solution (0.1% in PBS) for 10-15 min, the
stain was then removed by gentle rinsing under a tap. Stained monolayers were then
incubated with 150 µL per well of 1% SDS solution in PBS to solubilise the stain, over a
2h incubation period on an orbital shaker at room temperature. The resulting optical
density was measured at 560nm using a SpectraMax plate reader (Molecular Devices,
30 U.S.).

Lung virus load

RNA was extracted from whole lung tissue using RNeasy® mini kit columns according to manufacturers' instructions and converted to cDNA using Omniscript® Reverse
35 Transcription Kit (both Qiagen, UK). RV was quantified relative to 18S rRNA
expression using a 50 nM forward primer (5'-tgagtctccggcccctgaatg-3'), 300 nM

forward primer (5'-gtgaagagccscrtgtgct-3') and 5 μ M QuantiTect Probe (Qiagen) and interpolated using an RV plasmid standard generated in house. Quantitative rtPCR was performed using a QuantStudio 5 real time qPCR machine (ThermoFisher Scientific).

5 Ex vivo cell stimulations for flow cytometry

Single cell suspensions of lung or BAL cells were stimulated for 4 hours at 37°C in R10 medium containing 40 ng/mL PMA (Invivogen, U.S.) and 3 μ g/mL ionomycin (Stemcell Technologies, Canada), or 16 μ g/mL RV peptide pools, in the presence of 10 μ g/mL brefeldin A (Enzo Life Sciences, U.S.). Unstimulated control samples were
10 incubated in R10 medium containing brefeldin A alone.

Flow cytometry

Single cell suspensions of lung, BAL or lymph node cells were initially stained with Fixable Violet Dead Cell Stain Kit (Invitrogen, US), then stained with anti-mouse
15 surface marker antibodies plus TruStain FcX antibody diluted in FACS buffer (PBS containing 2% FCS and 2 mM EDTA (Fisher Scientific)) for 30–60 min. Cell were washed twice with FACS buffer and fixed with a 1% formaldehyde solution (Sigma-Aldrich, UK) in FACS buffer. For intranuclear staining, cells were fixed with
Transcription Factor Staining Buffer Set (eBioscience, US) and stained with anti-mouse
20 transcription factor and cytokine antibodies diluted in permeabilisation buffer (eBioscience) for 1 hour. Samples were acquired using an LSRFortessa (BD, US) and analysed using FlowJo™ (BD).

ELISA

25 Nunc Maxisorp 96-well plates (Fisher Scientific) were coated with 50 ng/well mouse anti-IgG antibody (R&D Systems, U.S.) diluted in PBS for the standard curve, and 25 μ g/well RV-A16, RV-Bo6 or RV-C24 protein diluted in PBS for detection of antigen-specific IgG in mouse serum samples. Plates were incubated overnight at 4°C. Plates were washed three times with PBS-Tween (PBS containing 0.05% Tween 20 (Sigma-Aldrich)). Non-specific binding was blocked by incubation with PBS 5% milk (Sigma-Aldrich) for 1 hour at room temperature. Plates were washed three time with PBS-Tween. 50 μ L standard or serum sample were added per well, diluted in PBS 5% milk and incubated for 2 hours at room temperature. A 7-point standard curve was
30 generated using serial 1:3 dilutions of Total IgG mouse ELISA standard (Invitrogen) starting from 100 μ g/mL. Plates were washed three times with PBS-Tween. Bound
35 antibody was detected with 25 ng/well Mouse IgG Biotinylated Antibody (R&D

Systems) diluted in PBS 1% BSA (Sigma-Aldrich), incubated for 1 hour at room temperature. Plates were washed three times with PBS-Tween, then incubated with 50 ng/well Streptavidin-HRP conjugate (Millipore, U.S.) for 20 minutes at room temperature in the dark. Plates were washed three times with PBS-Tween, then developed using 50 μ L TMB substrate (Fisher Scientific) per well incubated for approximately 5 minutes, followed by 50 μ L 0.18M H₂SO₄ per well to stop the reaction. Plates were read immediately at 450nm using a Petromax plate reader (Molecular Devices).

Results

Identification of centroid RV sequences to use as cross-protective immunogens

Defining thresholds for centroid identification

A study by Glanville et al (2013) [6], demonstrated that immunisation of mice with the VPO protein from RV-A16 generated cellular immunity that cross-reacted with RV-B14, RV-A1 and RV-A29. Identity scores for RV-B14, RV-A1 and RV-A29 VPO sequences as compared pairwise to the RV-A16 VPO sequence were calculated (Table 3).

Table 3 – Sequence and fragment identity and similarity scores from MUSCLE analysis of 150 RV strain VPO protein sequences used to demonstrate cross-immunisation in the Glanville et al. (2013) study. The threshold scores are highlighted in bold.

Matrix	Score (%)			
	RV-A16 VPO	RV-B14 VPO	RV-A1 VPO	RV-A29 VPO
Sequence identity to RV-A16 VPO	100	59.9	87.7	82.7
Sequence similarity to RV-A16 VPO	100	84.0	94.6	92.5
Fragment identity to RV-A16 VPO	100	3.1	48.3	29.6
Fragment similarity to RV-A16 VPO	100	72.9	90.0	86.1

Glanville et al (2013) [6] demonstrated that RV-A16 VPO protein immunisation of mice induces cellular immunity that cross-reacts with RV-A14, RV-A1 and RV-A29. The lowest identity and similarity scores were used as the thresholds: any VPO proteins with sequence identity scores of >59.9% or similarity scores of >84.0% (Table 3) were

predicted to generate cross-reactive cellular immunity against each other. Any VPO proteins with identity scores below these thresholds were not predicted to generate cross-reactive immunity to each other.

- 5 The thresholds were used to select centroid VPO proteins from each cluster.

Identification of centroids

Identification of centroids was first performed using sequence identity scores only as this is the most rigid approach.

10

Figure 1 is a heatmap of the MUSCLE computed sequence identity scores between the 150 RV strain VPO sequences. Those sequences with an identity score >59.9% are shown in blue. RV-A16 VPO has previously been demonstrated as a potential centroid VPO sequence for RV-A species. This data confirms that RV-A16 VPO sequence has
15 identity scores >59.9% against all other RV-A species strains and is therefore predicted to induce cellular immunity against all other A strains and was selected as the RV-A species centroid immunogen.

However, the heatmap demonstrates that RV-A16 VPO is not predicted to cross-
20 immunise against many RV-B and C species strains. Therefore, to identify additional RV VPO protein centroids that are predicted to induce cellular immunity against all other strains, hierarchical clustering based upon average linkage was performed. Figure 2 is a circular tree created by hierarchical clustering of 140 RV strain VPO sequences based on identity scores and split into three clusters. Cluster 1 (red) contains all RV-A
25 species sequences, cluster 2 (black) contains all RV-B species strains and cluster 3 (green) contains all RV-C strains included in the analysis. Based upon this analysis RV-Bo6 VPO was selected as the RV-B species centroid as it has identity scores against all other RV-B species VPO protein sequences above the threshold of 59.9% (Table 4). The RV-C species centroid was identified as RV-Co7, as this VPO sequence was calculated to
30 cover all other RV-C species VPO protein sequences above the threshold of 59.9% (Table 4).

*Table 4 – Percentage of RV strains within cluster 2 (n=25) and cluster 3 (n=47) predicted to be cross-immunised by RV-Bo6 VPO or RV-Co7 VPO at different sequence
35 identity thresholds. The required sequence identity threshold predicted for cross-immunisation is highlighted in bold.*

- 54 -

Sequence identity score threshold	% of strains within the cluster predicted to be cross-immunised by the centroid	
	Cluster 2 - RV-Bo6 VPo	Cluster 3 - RV-Co7 VPo
30%	100	100
40%	100	100
50%	100	100
60%	100	100
70%	100	100
80%	87.01	32.61
90%	11.69	4.35
95%	1.3	2.17

Identification of additional RV-C species centroids

The inventors then investigated the number of IFN- γ producing splenocytes from mice immunised with RV-A16 VPo or RV-Bo6 VPo protein. The data presented in Figures 3 and 4 demonstrates that, when used as immunogens in mice, RV-A16 VPo and RV-Bo6 VPo proteins, respectively, induce cellular cross-immunity against all other RV-A and RV-B species, respectively (Figures 3B and 4B). However, neither RV-A16 or RV-Bo6 VPo immunization induced cellular immunity to RV-C species (Figures 3C and 4C).

When RV-Co7 VPo was used as an immunogen, the splenocytes from mice immunised with RV-Co7 responded weakly to stimulation with peptide pools from other RV-C species strains (Figure 5B), compared to the responses of splenocytes from RV-A16 and RV-Bo6 immunised mice to stimulation with peptides from their respective species (Figures 3B and 4B). Furthermore, there was very little cross-reactivity with peptide pools from RV-A and B species strains (Figure 5C). Therefore, further analysis was performed to select new RV-C species VPo immunogens that would provide good cross-immunity against other RV-C species, but were dissimilar to RV-Co7.

A deeper level of analysis was required to identify RV-C centroids that were dissimilar from RV-Co7, but still predicted to cross-react with all other RV-C species strains. Therefore, the new analysis was performed using all scores calculated: sequence identity, sequence similarity, fragment identity and fragment similarity.

The identity and similarity scores were aggregated as the geometric mean of the logged measures (+1 was added before taking the log to avoid negative values). This value was

referred to as the identity/similarity score, with a higher score indicating higher similarity and identity. Two identity/similarity scores were reported, one for the whole VPo sequence analysis, and one for the fragment analysis: the sequence identity/similarity scores were used to compare against RV-Co7 VPo, and the fragment
 5 identity/similarity scores were used to predict the potential for cross-immunisation based upon the thresholds calculated previously.

In Figure 6, the median fragment identity/similarity score for each RV-C strain VPo sequence across all other RV-C VPo strains was plotted against the median sequence
 10 identity/similarity score for each strain against RV-Co7 alone. When all RV-C species strains were analysed as a single cluster (Figure 6A), RV-C24, C25 and C49 VPo had the highest median fragment identity/similarity score across all other RV-C species strains tested, while having a sequence identity/similarity score that was different from RV-Co7. When the RV-C species strains were split into two clusters (Figure 6B) RV-C19
 15 VPo, was identified as a potential centroid from the 1st cluster and RV-Co1 VPo from the second cluster.

When the VPo sequences identified were compared using the non-aggregated sequence identities and similarities to RV-Co7 VPo and the non-aggregated identities and
 20 sequences to all RV-C strains VPo (Table 5), RV-C24 VPo was selected as the centroid RV-C species VPo, having the best overall balance between predicted cross-strain coverage and dissimilarity to RV-Co7 VPo. From the two-cluster analysis, RV-Co1 and RV-C19 VPo were selected for further testing.

25 *Table 5 – Sequence identity and similarity scores to RV-Co7 and fragment identity and similarity scores across all other RV-C species strains tested (n=47). The centroids selected are highlighted in bold.*

	RV-C24 VPo	RV-C25 VPo	RV-C49 VPo	RV-C19 VPo
Sequence identity to RV-Co7 VPo (%)	79.9	75.3	80.2	73.3
Sequence similarity to RV-Co7 VPo (%)	93.3	92.2	93.6	91.2
Fragment identity to all RV-C strains VPo (%)	14.2	11.2	14.2	5.7
Fragment similarity to all RV-C strains VPo (%)	86.2	86.9	87.1	89.3

RV-A16 VPo immunisation

Figure 3 is a set of histograms that show the results from ELISPOT experiments to analyse the cellular immunity evoked by immunisation with RV-A16 VPo protein against VPo from strains of other RV species. Mice were immunised twice with RV-A16 VPo protein with adjuvant and the spleens subsequently harvested, as described above. To quantify the level of cellular immunity evoked by immunisation with RV-A16 VPo, splenocytes were isolated and incubated with different stimuli, as indicated in the Figure 3. Splenocytes that are activated by the stimulus secrete the cytokine IFN- γ and the degree of IFN- γ secretion was quantified by ELISPOT analysis.

The results show that splenocytes from RV-A16 VPo immunised mice secrete IFN- γ in response stimulation with VPo peptide pools from a diverse set of A species RVs (Figure 3B). This is in addition to IFN- γ secretion in response to the restimulation with the same recombinant RV-A16 VPo protein used to immunise (Figure 3A).

When splenocytes from RV-A16 VPo immunised mice were stimulated with nothing (medium alone) or an irrelevant peptide, there was negligible production of IFN- γ by the splenocytes (Figures 3A and 3B). When the degree of IFN- γ secretion by splenocytes in response to RV-A species peptide pools was statistically compared to that produced in response to stimulation with the irrelevant peptide, the results for all peptide pools tested were highly significant (Figure 3B). These results demonstrate that immunisation of mice with RV-A16 VPo evokes cellular immunity that is cross-reactive with other members of the A species of RVs. However, when splenocytes from RV-A16 immunised mice were stimulated with peptide pools corresponding to VPo proteins from RV-B and C species strains, no significant secretion of IFN- γ was observed (Figure 3C). This indicates that RV-A16 immunisation does not induce cross-reactive cellular immunity to strains outside of RV-A species.

RV-Bo6 VPo immunisation

Figure 4 is a set of histograms that show the results from ELISPOT experiments to analyse the cellular immunity evoked by immunisation with RV-Bo6 VPo protein. Mice were immunised twice with RV-Bo6 VPo protein with adjuvant and the spleens subsequently harvested, as described above. To quantify the level of cellular immunity evoked by immunisation with RV-Bo6 VPo, splenocytes were isolated and incubated with different stimuli, as indicated in Figure 4. Splenocytes that are activated by the

stimulus secrete the cytokine IFN- γ and the degree of IFN- γ secretion was quantified by ELISPOT analysis.

5 The results show that splenocytes from RV-Bo6 VPo immunised mice secrete IFN- γ in response stimulation with VPo peptide pools from a diverse set of B species RVs (Figure 4B). This is in addition to IFN- γ secretion in response to the immunising RV-Bo6 VPo protein (Figure 4A) and the RV-Bo6 peptide pool (Figure 4B).

10 When splenocytes from RV-Bo6 VPo immunised mice were stimulated with nothing (medium alone) or an irrelevant peptide, there was negligible production of IFN- γ by the splenocytes (Figures 4A and 4B). When the degree of IFN- γ secretion by splenocytes in response to RV-B species peptide pools was statistically compared to that produced in response to stimulation with the irrelevant peptide, the results for all peptide pools tested were highly significant (Figure 4B).

15 These results demonstrate that immunisation of mice with RV-Bo6 VPo evokes cellular immunity that is cross-reactive with other members of the B species of RVs. However, when splenocytes from RV-Bo6 immunised mice were stimulated with peptide pools corresponding to VPo proteins from RV-A and C species strains, significant secretion of
20 IFN- γ was only observed in response to peptides from some RV-A species strains (Figure 4C). This indicates that RV-Bo6 VPo immunisation induces limited cross-reactive cellular immunity to RV-A species strains, and none to RV-C species.

To further demonstrate that immunisation of mice with RV-Bo6 VPo induces
25 immunity, the humoral response to the immunising antigen was assessed. Mice were immunised twice with RV-Bo6 VPo protein with adjuvant and the serum subsequently harvested, as described above. The quantity of RV-Bo6 VPo specific antibodies in the mouse serum was determined by ELISA. Figure 10 is a set of line graphs that shows the levels of anti-RV-Bo6 VPo antibodies in serum from mice following immunisation, as
30 quantified by ELISA.

In all mice immunised with RV-Bo6 VPo, there are high levels of antibody that specifically recognise RV-Bo6 VPo, while in control immunised mice there are no discernible levels. These results demonstrate that, in addition to the induction of cross-
35 reactive cellular immunity by immunisation with RV-Bo6 VPo, there is also a strong humoral response to the immunising protein.

RV-Co7 VPo immunisation

Figure 5 is a set of histograms that show the results from ELISPOT experiments to analyse the cellular immunity evoked by immunisation with RV-Co7 VPo protein against VPo peptides from other strains of RV. Mice were immunised twice with RV-Co7 VPo protein with adjuvant and the spleens subsequently harvested, as described above. To quantify the level of cellular immunity evoked by immunisation with RV-Co7 VPo, splenocytes were isolated and incubated with different stimuli, as indicated in Figure 5. Splenocytes that are activated by the stimulus secrete the cytokine IFN- γ and the degree of IFN- γ secretion was quantified by ELISPOT analysis.

The results show that while splenocytes from RV-Co7 VPo immunised mice secrete IFN- γ when re-stimulated with the RV-Co7 VPo immunogen (Figure 5A), there is less significant IFN- γ secretion in response to stimulation with peptide pools corresponding to other RV-C species strains (Figure 5B). Furthermore, there was very little cross-reactivity to VPo peptides from other RV species (Figure 5C). As a result, deeper bioinformatical analysis was performed to identify other VPo sequences that could act as centroid immunogens to cover RV-C species strains.

RV-Co1 VPo immunisation

Figure 7 is a set of histograms that show the results from ELISPOT experiments to analyse the cellular immunity evoked by immunisation with RV-Co1 VPo protein against other strains of RV. Mice were immunised twice with RV-Co1 VPo protein with adjuvant and the spleens subsequently harvested, as described above. To quantify the level of cellular immunity evoked by immunisation with RV-Co1 VPo, splenocytes were isolated and incubated with different stimuli, as indicated in Figure 7. Splenocytes that are activated by the stimulus secrete the cytokine IFN- γ and the degree of IFN- γ secretion was quantified by ELISPOT analysis.

The results show that splenocytes from RV-Co1 VPo immunised mice secrete IFN- γ in response to stimulation with VPo peptide pools from a diverse set of C species RVs (Figure 7B). This is in addition to IFN- γ secretion in response to the immunising RV-Co1 VPo protein (Figure 7A) and peptide pool (Figure 7B).

When splenocytes from RV-Co1 VPo immunised mice were stimulated with nothing (medium alone; Figure 7A) or an irrelevant peptide (Figure 7B), there was negligible

production of IFN- γ by the splenocytes. When the degree of IFN- γ secretion by splenocytes in response to RV-C species peptide pools was statistically compared to that produced in response to stimulation with the irrelevant peptide, the results for all peptide pools tested, except RV-C19 VPo peptides, were highly significant (Figure 7B).

5

These results demonstrate that immunisation of mice with RV-Co1 VPo evokes cellular immunity that is cross-reactive with other members of the C species of RVs.

Furthermore, when splenocytes from RV-Co1 immunised mice were stimulated with VPo peptide pools from strains of other RV-species, there was significant IFN- γ

10 secretion in response to peptides from both RV-A and B species (Figure 7C).

To further demonstrate that immunisation of mice with RV-Co1 VPo induces immunity, the humoral response to the immunising antigen was assessed. Mice were immunised twice with RV-Co1 VPo protein with adjuvant and the serum subsequently
15 harvested, as described above. The quantity of RV-Co1 VPo specific antibodies in the mouse serum was determined by ELISA. Figure 10 is a set of line graphs that shows the levels of anti-RV-Co1 VPo antibodies in serum from mice following immunisation, as quantified by ELISA.

20 In all mice immunised with RV-Co1 VPo there are high levels of antibody that specifically recognises RV-Co1 VPo, while in control immunised mice there are no discernible levels. These results demonstrate that, in addition to the induction of cross-reactive cellular immunity by immunisation with RV-Co1 VPo, there is also a strong humoral response to the immunising protein.

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RV-C19 VPo immunisation

Figure 8 is a set of histograms that show the results from ELISPOT experiments to analyse the cellular immunity evoked by immunisation with RV-C19 VPo protein against other strains of RV. Mice were immunised twice with RV-C19 VPo protein with
30 adjuvant and the spleens subsequently harvested, as described above. To quantify the level of cellular immunity evoked by immunisation with RV-C19 VPo, splenocytes were isolated and incubated with different stimuli, as indicated in Figure 8. Splenocytes that are activated by the stimulus secrete the cytokine IFN- γ and the degree of IFN- γ secretion was quantified by ELISPOT analysis.

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- 60 -

The results show that splenocytes from RV-C19 VPo immunised mice secrete IFN- γ in response to stimulation with VPo peptide pools from a diverse set of C species RVs (Figure 8B). This is in addition to IFN- γ secretion in response to the immunising RV-C19 VPo protein (Figure 8A) and peptide pool (Figure 8B).

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When splenocytes from RV-C19 VPo immunised mice were stimulated with nothing (medium alone; Figure 8A) or an irrelevant peptide (Figure 8B), there was negligible production of IFN- γ by the splenocytes. When the degree of IFN- γ secretion by splenocytes in response to RV-C species peptide pools was statistically compared to that produced in response to stimulation with the irrelevant peptide, the results for all peptide pools tested were highly significant (Figure 8B).

These results demonstrate that immunisation of mice with RV-C19 VPo evokes cellular immunity that is cross-reactive with other members of the C species of RVs. Furthermore, when splenocytes from RV-C19 immunised mice were stimulated with VPo peptide pools from strains of other RV-species, there was significant IFN- γ secretion to in response to peptides from both RV-A and B species (Figure 8C).

To further demonstrate that immunisation of mice with RV-C19 VPo induces immunity, the humoral response to the immunising antigen was assessed. Mice were immunised twice with RV-C19 VPo protein with adjuvant and the serum subsequently harvested, as described above. The quantity of RV-C19 VPo specific antibodies in the mouse serum was determined by ELISA. Figure 10 is a set of line graphs that shows the levels of anti-RV-C19 VPo antibodies in serum from mice following immunisation, as quantified by ELISA.

In all mice immunised with RV-C19 VPo there are high levels of antibody that specifically recognises RV-C19 VPo, while in control immunised mice there are no discernible levels. These results demonstrate that, in addition to the induction of cross-reactive cellular immunity by immunisation with RV-C19 VPo, there is also a strong humoral response to the immunising protein.

RV-C24 VPo immunisation

Figure 9 is a set of histograms that show the results from ELISPOT experiments to analyse the cellular immunity evoked by immunisation with RV-C24 VPo protein against other strains of RV. Mice were immunised twice with RV-C24 VPo protein with

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- 61 -

adjuvant and the spleens subsequently harvested, as described above. To quantify the level of cellular immunity evoked by immunisation with RV-C24 VPo, splenocytes were isolated and incubated with different stimuli, as indicated in Figure 9. Splenocytes that are activated by the stimulus secrete the cytokine IFN- γ and the degree of IFN- γ secretion was quantified by ELISPOT analysis.

The results show that splenocytes from RV-C24 VPo immunised mice secrete IFN- γ in response to stimulation with VPo peptide pools from a diverse set of C species RVs (Figure 9B). This is in addition to IFN- γ secretion in response to the immunising RV-C24 VPo protein (Figure 9A) and the RV-C24 peptide pool (Figure 9B).

When splenocytes from RV-C24 VPo immunised mice were stimulated with nothing (medium alone; Figure 9A) or an irrelevant peptide (Figure 9B), there was negligible production of IFN- γ by the splenocytes. When the degree of IFN- γ secretion by splenocytes in response to RV-C species peptide pools was statistically compared to that produced in response to stimulation with the irrelevant peptide, the results for all peptide pools tested were highly significant (Figure 9B).

These results demonstrate that immunisation of mice with RV-C24 VPo evokes cellular immunity that is cross-reactive with other members of the C species of RVs. Furthermore, when splenocytes from RV-C24 immunised mice were stimulated with VPo peptide pools from strains of other RV-species, there was significant IFN- γ secretion to in response to all peptides from both RV-A and B species (Figure 9C). This suggests that RV-C24 VPo has the potential to induce cross-reactive cellular immunity against all species of RV.

To further demonstrate that immunisation of mice with RV-C24 VPo induces immunity, the humoral response to the immunising antigen was assessed. Mice were immunised twice with RV-C24 VPo protein with adjuvant and the serum subsequently harvested, as described above. The quantity of RV-C24 VPo specific antibodies in the mouse serum was determined by ELISA. Figure 10 is a set of line graphs that shows the levels of anti-RV-C24 VPo antibodies in serum from mice following immunisation, as quantified by ELISA.

In all mice immunised with RV-C24 VPo there are high levels of antibody that specifically recognises RV-C24 VPo, while in control immunised mice there are no

- 62 -

discernible levels. These results demonstrate that, in addition to the induction of cross-reactive cellular immunity by immunisation with RV-C24 VPo, there is also a strong humoral response to the immunising protein.

5 Recombinant RV-C24 VPo evoked immunogenicity in cynomolgus monkeys

Cynomolgus monkeys were immunised twice with recombinant RV-C24 VPo (rRV-C24 VPo) protein with adjuvant or adjuvant alone (control), and blood was taken 28 days after the first immunization and PBMCs prepared, as described. To quantify the level of cellular immunity evoked by immunisation with rRV-C24 VPo protein, PBMCs were
10 incubated with different peptide pool stimuli, corresponding to full-length VPo from different strains of RV or controls, as indicated on the x axes in Figure 11. PBMCs that are activated by the stimulus secrete the cytokine IFN- γ and the degree of IFN- γ secretion was quantified by ELISPOT analysis.

15 The results show that PBMCs from an rRV-C24 VPo immunised representative cynomolgus monkey secrete IFN- γ in response to stimulation with VPo peptide pools from a diverse set of A, B and C species RVs (Figure 11B). PBMCs from an animal immunized with adjuvant alone (control) produced negligible IFN- γ in response to VPo peptide pool stimulation (Figure 11A).

20

When PBMCs from an rRV-C24 VPo immunised cynomolgus monkey were stimulated with an irrelevant peptide control pool (Figure 11B), there was negligible production of IFN- γ . When PBMCs from a control cynomolgus monkey were stimulated with an irrelevant peptide control pool or with the VPo peptide pools (Figure 11A), the
25 production of IFN- γ was again negligible.

These results demonstrate that immunisation of cynomolgus monkeys with rRV-C24 VPo evokes cellular immunity that is cross-reactive with strains from A, B and C species of RVs.

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To demonstrate the Th phenotype of the cellular immune response, the IFN- γ responses of PBMCs from two cynomolgus monkeys immunized with adjuvant alone (control) or rRV-C24 VPo protein, was compared to the IL-4 response by ELISPOT after stimulation with peptide pools. IFN- γ is a canonical Th1 cytokine and IL-4 is a
35 canonical Th2 cytokine. Figure 12A demonstrates that PBMCs from two cynomolgus monkeys immunized with rRV-C24 VPo protein, but not adjuvant only (control),

- 63 -

secrete IFN- γ in response to stimulation with peptide pools from C species of RV that are greater than that produced in response to the irrelevant peptide control pool, but that IL-4 secretion is negligible (Figure 12B).

- 5 To further demonstrate that immunization with rRV-C24 VPo induces immunity in cynomolgus monkeys, the humoral response to the immunising antigen was assessed. Figure 13 is a histogram of RV-C24 VPo-binding IgG, as quantified by ELISA, in the plasma of cynomolgus monkeys at different timepoints after immunization with rRV-C24 VPo protein. The results show that immunization with rRV-C24 VPo evokes
10 production of RV-C24 VPo-specific IgG antibodies, which increase in magnitude over time, whereas animals immunized with the adjuvant alone (control) did not produce detectable RV-C24 VPo-specific IgG.

Transfection of HEK293 cells with RV VPo mRNA

- 15 To demonstrate that RV VPo mRNA is translated into full-length VPo protein in cells, HEK293 cells were transfected with different RV VPo mRNAs, as described above.

Figure 14A is a Western blot of lysates from HEK293 cells transfected with RV-A16 VPo mRNA made at 24, 48, 72 or 96 hours after transfection. A positive control of
20 recombinant RV-A16 VPo protein was loaded onto the gel (labelled rRV-A16 VPo). The results demonstrate that transfection of HEK293 cells with RV-A16 VPo mRNA results in the expression of RV-A16 VPo protein, which peaks at 48 hrs after transfection.

- Figure 14B is a Western blot of lysates from HEK293 cells transfected with RV-Bo6 or
25 RV-C24 VPo mRNA made at 24, 48, 72 or 96 hours after transfection. The results demonstrate that HEK293 cells transfected with RV-Bo6 VPo or C24 VPo mRNAs express RV-Bo6 and RV-C24 VPo protein, respectively. Expression of both proteins peaks at 24 and 48 hours after transfection. By comparing the VPo bands detected using the monoclonal antibody or antisera to the marker, it is observed that the VPo
30 bands are of the correct approximate molecular weight of 36 kDa.

RV VPo mRNA evoked immunogenicity in mice

- Mice were immunised twice with RV-C24 VPo mRNA, spleens harvested 42 days after the first immunization and splenocytes prepared, as described above. To quantify the
35 level of cellular immunity evoked by immunisation with RV-C24 VPo mRNA, splenocytes were incubated with peptide pools corresponding to full length VPo

- 64 -

proteins from different strains of RV or a control pool, as indicated in Figure 15. Splenocytes that are activated by the stimulus secrete the cytokine IFN- γ and the degree of IFN- γ secretion was quantified by ELISPOT analysis.

5 The results show that splenocytes from RV-C24 VPo mRNA immunised mice secrete IFN- γ in response to stimulation with VPo peptide pools from a diverse set of C species RVs. When splenocytes from RV-C24 VPo mRNA immunised mice were stimulated with an irrelevant peptide pool (Figure 15) there was negligible production of IFN- γ .
10 When the responses to the VPo peptide pools stimuli were statistically compared to the responses to the irrelevant peptide pool, the results were significant. These results demonstrate that immunisation of mice with RV-C24 VPo mRNA evokes cellular immunity that is cross-reactive with other members of C species RVs.

To further demonstrate that immunization with RV-C24 VPo mRNA induces immunity
15 in mice, the humoral response to the immunising antigen was assessed. Figure 16 is a histogram showing the concentrations of RV-C24 VPo-binding IgG, as quantified by ELISA, in the serum of mice at different timepoints after immunization with RV-C24 VPo mRNA. The results show that immunization with RV-C24 VPo mRNA evokes production of RV-C24 VPo-specific IgG antibodies, which increase in magnitude over
20 time, whereas control animals did not produce detectable RV-C24 VPo-specific IgG.

RV VPo mRNA evoked immunogenicity in cynomolgus monkeys

Figure 17 is a histogram that show the results from ELISPOT experiments to analyse the cellular immunity evoked by immunisation with an mRNA concatemer consisting of
25 RV-C24/A16/Bo6 VPo mRNAs in sequence separated by a 2A peptide-encoding sequence. Immunizing animals with the RV VPo mRNA concatemer allows the immune responses to each antigen to be determined.

Cynomolgus monkeys were immunised twice with the RV-C24/A16/Bo6 VPo mRNA
30 concatemer or control, blood was taken 28 days after the first immunization and PBMCs prepared, as described above. To quantify the level of cellular immunity to each antigen evoked by immunisation with the RV-C24/A16/Bo6 VPo mRNA concatemer, PBMCs were incubated with peptide pools corresponding to full-length RV-A16, Bo6, C24 VPo, or a control irrelevant peptide pool and IFN- γ (Figure 17A) or IL-4 (Figure
35 17B) secretion quantified by ELISPOT.

- 65 -

The results show that PBMCs from RV-C24/A16/Bo6 concatemer VPo mRNA immunised cynomolgus monkeys secrete IFN- γ in response to stimulation with VPo peptide pools corresponding to full-length RV-A16, Bo6 and C24 VPo. When PBMCs from RV-C24/A16/Bo6 concatemer VPo mRNA immunised monkeys were stimulated with an irrelevant peptide pool there was negligible production of IFN- γ . PBMCs from control immunized animals produced negligible IFN- γ in response to stimulation with RV VPo peptide pools or the irrelevant peptide control pool. The results in Figure 17A demonstrate that immunisation of cynomolgus monkeys with RV-C24/A16/Bo6 concatemer VPo mRNA evokes cellular immunity to each of the three antigens produced by the VPo mRNA concatemer.

To demonstrate the Th phenotype of the cellular immune response, the IFN- γ response from PBMCs from cynomolgus monkeys immunized with RV-C24/A16/Bo6 VPo mRNA concatemer was compared to the IL-4 response by ELISPOT. IFN- γ is a canonical Th1 cytokine and IL-4 is a canonical Th2 cytokine. Figure 17B demonstrates that PBMCs from cynomolgus monkeys immunized with RV-C24/A16/Bo6 concatemer VPo mRNA produce negligible IL-4 compared to IFN- γ (Figure 17A) in response to stimulation with RV VPo or control peptide pools. This result demonstrates that the cellular immune response primed by RV-C24/A16/Bo6 concatemer VPo mRNA is Th1-polarized.

To further demonstrate that immunisation of cynomolgus monkeys with RV VPo mRNA induces immunity, the humoral responses to each antigen produced by the RV-C24/A16/Bo6 VPo mRNA concatemer was determined.

Figure 18 is a histogram showing the concentrations of RV-A16 (Figure 18A), RV-Bo6 (Figure 18B) and RV-C24 (Figure 18C) VPo-binding IgG, as quantified by ELISA, in the plasma of cynomolgus monkeys at different timepoints after immunization. These results show that immunization with the RV-C24/A16/Bo6 VPo mRNA concatemer evokes production of RV-VPo-specific IgG antibodies to each of the three RV antigens in the concatemer in cynomolgus monkeys.

Efficacy of RV VPo mRNA immunization in mice

To demonstrate that immunization with RV VPo mRNA is protective against subsequent heterotypic RV infection, mice were immunized with RV-A16 VPo mRNA

- 66 -

and then infected intranasally with RV-A1, a heterotypic A species strain of RV to the immunizing strain. The methods are described above.

Figure 19 is a histogram that shows the concentration of RV-A1 RNA in the lungs of control and RV-A16 VPo mRNA immunized mice, as determined by RT-qPCR. The results demonstrate that while control and immunized mice have similar levels of viral RNA in the lungs one day after infection, by day six the immunized mice have significantly less viral RNA than control mice. By day 14, the concentration of viral RNA is negligible in both groups of animals. This result demonstrates that immunization with RV VPo mRNA accelerates the clearance of a heterotypic RV infection from the lungs of mice.

RV VPo mRNA evoked cellular immunity

To demonstrate how immunization with RV VPo mRNA protects mice against RV infection, the inventors harvested spleens from mice that had been immunized with RV-A16 VPo mRNA or control and then infected with heterotypic RV-A1, at 14 days after infection and created splenocytes. The splenocytes were stimulated with peptide pools corresponding to full-length VPo from a diverse set of strains covering each species of RV or a control irrelevant peptide pool. IFN- γ secretion in response to peptide stimulation was measured by ELISPOT.

The results shown in Figure 20 were surprising and demonstrate that splenocytes from mice immunized with RV-A16 VPo mRNA and then infected with RV-A1 secrete IFN- γ in response to stimulation with VPo peptide pools from a wide range of RV strains, covering A, B and C species. When the results obtained with VPo peptide pools were statistically compared to those obtained with the irrelevant peptide control pool, the results were highly significant.

In contrast, as shown in Figure 21, in the control mice, there were negligible IFN- γ secreting splenocytes after stimulation with RV VPo or control peptide pools. The results in Figures 20 and 21 demonstrate that immunization with VPo mRNA from a single strain of RV produces surprising cross-strain cellular immunity.

To further demonstrate the mechanism by which RV VPo mRNA immunization protects mice from infection with RV-A1, the inventors performed immunophenotyping

- 67 -

on the lungs of RV-A16 VPo mRNA immunized and non-immunized mice after infection with RV-A1. The methods are described above.

Figure 22 is a histogram that shows the mean numbers of CD62⁻ CD44⁺ effector CD4⁺ (Figure 22A) and CD8⁺ (Figure 22B) T cells, respectively, in the lungs of RV-A16 VPo mRNA immunized and control mice after infection with RV-A1. The results demonstrate that there are significantly more effector CD4⁺ and CD8⁺ T cells in the lungs of immunized mice 6 and 14 days after infection than in control mice.

10 RV VPo mRNA immunization primes Th1-polarized T cell immunity

To determine the Th phenotype primed by RV VPo mRNA immunization, lungs were harvested from mice that had been infected with RV-A1 after immunization with RV-A16 VPo mRNA or control. A single cell population was prepared from the lungs and the cells incubated with a peptide pool corresponding to full-length VPo from RV-A1. Intracellular cytokine staining flow cytometry analysis was performed to measure the frequency of Th1 and Th2 cells, as described.

Th1 cells were identified as T-bet⁺ IFN- γ ⁺ CD4⁺ cells and Th2 cells were identified as Gata3⁺ IL-4⁺ CD4⁺ cells. Figure 23 is a set of correlation plots where the frequency of Th1 cells is plotted against the frequency of Th2 cells for each animal after stimulation of the lung cells with RV-A1 VPo peptide pool.

The results demonstrate that the frequency of RV-A1 VPo reactive Th1 cells is greater than RV-A1 VPo reactive Th2 cells in the lungs of immunized mice (Figure 23B). In control mice (Figure 23A), the opposite is observed, where the proportion of Th2 cells is greater than Th1 cells. This result demonstrates that RV VPo mRNA primes a Th1-polarized T cell response, with a greater proportion of RV-reactive CD4⁺ T cells of the Th1 phenotype than the Th2 phenotype in the lungs of immunized mice.

30 RV VPo primes tissue resident T cell memory

A critical component of protective cellular immunity elicited by natural infection with respiratory viruses is the formation of tissue resident memory T cells (T_{RM}) in the airway mucosa and lungs. These T_{RM} cells rapidly expand and clear subsequent infections.

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- 68 -

- To determine if an intramuscularly delivered RV VPo mRNA vaccine is able to prime the formation of T_{RM} cells in the airways and lungs of immunized mice, bronchoalveolar lavage (BAL) and lungs were harvested from mice that had been infected with RV-A01 after immunization with RV-A16 VPo mRNA or control. A single cell population was prepared from the lungs and immunophenotyping performed to identify and enumerate CD4⁺ and CD8⁺ T_{RM} cells at different post-infection timepoints. CD4⁺ and CD8⁺ T_{RM} cells were identified as CD103⁺ CD69⁺ CD4⁺ or CD103⁺ CD69⁺ CD8⁺ cells, respectively.
- Figure 24 is a set of histograms showing the number of CD4⁺ (Figure 24A) and CD8⁺ (Figure 24B) T_{RM} cells in the BAL of control and RV-A16 VPo mRNA immunized mice at different post-RV-A1 infection timepoints. The results show that there are significantly more CD4⁺ and CD8⁺ T_{RM} cells in the BAL of immunized mice six days after infection compared to control mice.
- Figure 25 is a set of histograms showing the frequency of CD4⁺ (Figure 25A) and CD8⁺ (Figure 25B) T_{RM} cells in the BAL of control and RV-A16 VPo mRNA immunized mice at different post-RV-A1 infection timepoints. The results show that CD4⁺ and CD8⁺ T_{RM} cells are present at a significantly higher proportion of total CD4⁺ or CD8⁺ cells, respectively, in the BAL of immunized mice six and fourteen days after infection compared to control mice.
- Figure 26 is a set of histograms showing the number of CD4⁺ (Figure 26A) and CD8⁺ (Figure 26B) T_{RM} cells in the lungs of control and RV-A16 VPo mRNA immunized mice at different post-RV-A1 infection timepoints. The results show that there are significantly more CD4⁺ T_{RM} cells six days after infection and significantly more CD8⁺ T_{RM} cells six and fourteen days after infection in the lungs of immunized compared to control mice.
- Figure 27 is a set of histograms showing the frequency of CD4⁺ (Figure 27A) and CD8⁺ (Figure 27B) T_{RM} cells in the lungs of control and RV-A16 VPo mRNA immunized mice at different post-RV-A1 infection timepoints. The results show that CD4⁺ and CD8⁺ T_{RM} cells are present at a significantly higher proportion of total CD4⁺ or CD8⁺ cells, respectively, in the lungs of immunized mice six and fourteen days after infection compared to control mice.

- 69 -

These results demonstrate that intramuscular administration of a RV VPo mRNA vaccine primes T_{RM} cells in the airways and lungs of mice. Upon subsequent infection with a heterotypic strain of RV, the vaccine primed T_{RM} cells rapidly expand.

5 Conclusions

The inventors have identified RV VPo proteins from single representative strains, which are able to elicit broad cellular immune responses that cross-react with other RV strains from the same species. In particular, as illustrated in the Examples (Figures 3, 4, 5, 7, 8 and 9) splenocytes from RV-A16 immunised mice cross-react with VPo
10 peptides from other RV-A species strains, RV-Bo6 VPo immunised mice cross react with VPo peptides from other RV-B species members and peptides from some RV-A species strains, and splenocytes from either RV-Co1, RV-C19 or RV-C24 VPo immunised mice cross-react with peptides from other RV-C species members. Additionally, immunisation with VPo protein from RV-C species confers cellular
15 immunity that recognises VPo peptides from both RV-A and RV-B species. Accordingly, an immunogenic composition according to the claimed invention, comprising VPo peptides selected from RV-A16, RV-Bo6, RV-Co1, RV-C19 and RV-C24, in any combination, can be used to immunise humans against infection with all RV strains.

20 Additionally, the inventors have demonstrated that immunisation with mRNA encoding the RV VPo peptides, elicits a broad cellular immune response. For example, as shown in Figure 15, the inventors demonstrated that immunisation of mice with RV-C24 VPo mRNA evokes cellular immunity that is cross-reactive with other members of C species of RV.

25 Advantageously, therefore, the immunogenic composition according to the invention overcomes the issue of antigenic heterogeneity across RV strains, which has hampered RV vaccine development to date. Furthermore, the immunogenic composition is particularly effective, as vaccination with RV VPo antigens will provide protection for
30 patients who have chronic lung conditions and are therefore at risk of RV-induced exacerbation of their conditions, thereby reducing the associated morbidity, mortality and healthcare burden.

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- 70 -

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Claims

1. An immunogenic composition comprising at least one isolated human rhinovirus (RV) VPo peptide, or an isolated polynucleotide encoding the peptide,
5 wherein the peptide is selected from a group consisting of: an RV-C VPo peptide; an RV-B VPo peptide; RV-A28 VPo; and RV-A89 VPo, or a variant or fragment thereof.
2. The immunogenic composition according to claim 1, wherein the immunogenic composition comprises at least two isolated human rhinovirus (RV) VPo peptides, or
10 isolated polynucleotides encoding the peptides, wherein the peptides are selected from a group consisting of: an RV-C VPo peptide; an RV-B VPo peptide; RV-A28 VPo; and RV-A89 VPo.
3. The immunogenic composition according to either claim 1 or claim 2, wherein
15 the immunogenic composition comprises at least three isolated human rhinovirus (RV) VPo peptides, or isolated polynucleotides encoding the peptides, wherein the peptides are selected from a group consisting of: an RV-C VPo peptide; an RV-B VPo peptide; RV-A28 VPo; and RV-A89 VPo.
- 20 4. The immunogenic composition according to any preceding claim, wherein the RV-C VPo peptide is selected from a group consisting of: RV-Co7 VPo; RV-C19 VPo; RV-C24 VPo; and RV-Co1 VPo, or a variant or fragment thereof.
5. The immunogenic composition according to any preceding claim, wherein the
25 RV-B VPo peptide is RV-Bo6 VPo.
6. The immunogenic composition according to any preceding claim, wherein the immunogenic composition further comprises an isolated RV-A16 VPo peptide and/or an isolated RV-A29 VPo peptide, or an isolated polynucleotide encoding the peptide, or
30 a variant or fragment thereof.
7. The immunogenic composition according to any preceding claim, wherein the fragment of the at least one RV VPo peptide comprises or consists of the corresponding VP2 peptide and/or VP4 peptide.

- 72 -

8. The immunogenic composition according to any preceding claim, wherein at least two or more of the RV VPo peptides form a fusion peptide.
9. The immunogenic composition according to any preceding claim, wherein the
5 polynucleotide comprises a nucleic acid sequence encoding the peptide, preferably wherein the nucleic acid sequence is placed under the control of the elements necessary for its expression in a mammalian cell.
10. The immunogenic composition according to claim 9, wherein the nucleic acid is
10 DNA or RNA, optionally wherein the RNA is messenger RNA (mRNA) or self-amplifying RNA (saRNA).
11. The immunogenic composition according to any preceding claim, wherein the
15 isolated polynucleotide encodes at least two or more of the RV VPo peptides, or wherein at least two or more of the RV VPo peptides are encoded by a plurality of separate polynucleotides.
12. The immunogenic composition according to any preceding claim, wherein the
20 immunogenic composition comprises an isolated polynucleotide encoding the RV-C24 VPo peptide, an isolated polynucleotide encoding the RV-A16 VPo peptide, and an isolated polynucleotide encoding the RV-Bo6 VPo peptide.
13. The immunogenic composition according to any preceding claim, wherein the at
25 least one RV VPo peptide comprises an amino acid sequence substantially as set out in any of SEQ ID Nos: 1, 4, 7, 10, 13, 16, 19, 22 and/or 25, or a variant or fragment thereof.
14. The immunogenic composition according to any preceding claim, wherein the
30 isolated polynucleotide comprises a nucleotide sequence substantially as set out in any of SEQ ID Nos: 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 23, 24, 26 and/or 27, or a variant or fragment thereof.
15. The immunogenic composition according to any preceding claim, wherein the immunogenic composition further comprises an adjuvant.
- 35 16. The immunogenic composition according to any preceding claim, wherein the immunogenic composition is a vaccine.

- 73 -

17. The immunogenic composition according to any preceding claim, for use in therapy or prophylaxis.

5 18. The immunogenic composition according to any one of claims 1-16, for use in eliciting an immune response, preferably against a rhinovirus infection.

19. The immunogenic composition for use according to claim 18, wherein the immunogenic composition elicits an immune response against at least one, at least two,
10 or at least three species of rhinoviruses, more particularly, against RV-A, RV-B, and/or RV-C.

20. The immunogenic composition for use according to claim 18 or claim 19, wherein the immunogenic composition elicits an immune response against at least one
15 strain of RV-A, at least one strain of RV-B, and/or at least one strain of RV-C.

21. The immunogenic composition for use according to any one of claims 18-20, wherein the immunogenic composition elicits an immune response against common colds, virus-induced wheezing illnesses, exacerbations of asthma, bronchiectasis,
20 chronic obstructive pulmonary disease (COPD), cystic fibrosis and/or chronic fibrosing lung disease.

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