

COMPOSITIONS FOR USE IN TREATMENT OF CHLAMYDIA

FIELD OF THE INVENTION

The invention is in the field of treating and preventing *Chlamydia* infections. In particular, the invention relates to antigens and antigen combinations which can be used as vaccines to immunise against *Chlamydia* *sp.* infection. The vaccines may be delivered as nucleic acids (e.g., mRNAs) encoding antigenic proteins or as recombinant protein antigens.

BACKGROUND

Chlamydiae are intracellular bacterial pathogens responsible for a variety of infections, including the human sexually transmitted disease and eye infections (Trachoma) caused by *Chlamydia trachomatis*. The genus *Chlamydia* further comprises the species *Chlamydia abortus*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Chlamydia pecorum*, *Chlamydia felis*, and *Chlamydia caviae*.

Chlamydia trachomatis (*C. trachomatis*) comprises three biovars which lead to an array of pathological conditions in humans, and which are further subdivided into serovars.¹ Serovars A-C (A, B, Ba, and C), of the trachoma biovar, can cause Chlamydia conjunctivitis or trachoma, a disease that can result in blindness.² Serovars D-K (D, E, F, G, H, I, J, and K), of the genital tract biovar, cause disease in the genital tract.³ The lymphogranuloma venereum biovar, serovars L1-3 (L1, L2, and L3), causes invasive urogenital and anorectal infection and has become particularly associated with HIV-infected men who have sex with men.⁴

C. trachomatis (serovars D-K) is the most common bacterial agent of sexually transmitted infections.⁵ In 2020, the WHO estimated 129 million new *C. trachomatis* infections. These are of public health concern particularly because untreated infections are often asymptomatic or with minimal symptoms, but they contribute to the transmission of the pathogen. Furthermore, if left untreated, the infection can, amongst others, lead to salpingitis, endometritis, pelvic inflammatory disease (PID), ectopic pregnancy, tubal factor infertility, and can increase the risk for the transmission or acquisition of HIV and the development of cervical carcinoma.⁶ While *C. trachomatis* infections can be effectively controlled by antibiotic therapy, due to the high prevalence of cases and their frequently delayed diagnosis for asymptomatic cases, *C. trachomatis* has become a leading cause of female infertility worldwide.⁷ There is a need for developing an effective *C. trachomatis* vaccine as a sustainable strategy to curb infection rates of *C. trachomatis*.

DISCLOSURE OF THE INVENTION

The present inventors have provided *Chlamydia sp.* antigens and antigen combinations which can be used to immunise against *Chlamydia sp.*

In particular, the inventors found that antigens derived from a native major outer membrane protein (MOMP) polypeptide of *Chlamydia trachomatis* and *C. trachomatis* antigens other than MOMP elicited

robust T cell responses, particularly induction of IFN- γ -producing CD4+ T cells, and/or B cell (i.e. antibody) responses when delivered by mRNAs encoding the relevant antigens. In particular, T cell responses are considered critical for protective immunity.

Accordingly, the invention provides modified MOMP polypeptides, chimeric MOMP VD polypeptides and CT443, CT584, CT600 and CT812 *Chlamydia sp.* polypeptides and nucleic acids comprising a nucleotide sequence encoding such polypeptides.

Polypeptide antigens described herein may be delivered by, i.e. in the form of, a nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding said polypeptide.

MOMP

MOMP is an integral membrane porin protein⁸ found in bacteria of *Chlamydia sp.* It makes up roughly 60% of the protein mass in the *Chlamydia trachomatis* membrane⁹ and contains multiple B & T cell epitopes.¹⁰ In *C. trachomatis* infection, MOMP is an immunodominant antigen. Native MOMP polypeptides of *Chlamydia sp.* include five conserved domains separated by four surface exposed variable domains (VD1, VD2, VD3 and VD4).^{11,12} Thus, in a native MOMP polypeptide of *Chlamydia sp.*, VD1, VD2, VD3 and VD4 are surface exposed loops. Within *Chlamydia* species (e.g., *C. trachomatis*), native MOMP polypeptides vary in their VD sequences across different serovars.^{13,14} Within each serovar of *C. trachomatis*, native MOMP polypeptide sequences are highly conserved with greater than 98% sequence identity. Across serovars of *C. trachomatis*, the protein sequences are highly conserved, with exception of the surface exposed VDs. Neutralising antibody epitopes have been mapped to VDs.^{15,16,17,18,19}

The inventors have recognised that IFN- γ producing CD4+ T cells are critical for protective immunity; indeed cellular IFN- γ production has been shown to strongly correlate with protection²⁰ and CD4+ T cells appear to be critical since HIV+ women are at increased risk for chlamydia reinfection²¹. There is also evidence that *C. trachomatis* antibodies (i.e. B cell-mediated responses) alone may not prevent reinfection and the presence of anti- *C. trachomatis* IgG may even enhance the risk for incident infection.²² Anti-*C. trachomatis* antibodies have been shown to accelerate clearance of a primary *C. trachomatis* challenge in the presence of CD4+ T cells in mice, however, in the absence of CD4+ T cells, the primary challenge was not cleared.²³

A “native *Chlamydia sp.* MOMP polypeptide” as used herein encompasses a mature form of a full-length native MOMP polypeptide of a *Chlamydia sp.* without its native signal peptide sequence and a full-length native MOMP polypeptide of a *Chlamydia sp.* including its native signal peptide sequence. Preferably, the *Chlamydia sp.* referred to herein is *Chlamydia trachomatis*. A native *C. trachomatis* MOMP polypeptide may be of one of serovars A-C, D-K or L1-L3, e.g., serovars D-K. In some embodiments, the native *C. trachomatis* MOMP polypeptide is of one of serovars D-G. In some preferred embodiments, the native *C.*

trachomatis MOMP polypeptide is of serovar E. Exemplary sequences of native *Chlamydia sp.* MOMP polypeptides comprising the native signal peptide sequence are provided by SEQ ID NOs 1-4.

The amino acid sequence of a native *C. trachomatis* MOMP polypeptide of serovar E is provided in SEQ ID NO: 2:

5 MKKLLKSVLV FAALSSASSL QALPVGNPAE PSLMIDGILW EGFGGDPCDP CTTWCDAISM
 RMGYYGDFVF DRVLKTDVVK EFQMGDKPTS TTGNATAPTT LTARENPAYG RHMQDAEMFT
 NAACMALNIW DRFDVFCTLG ASSGYLKNS ASFNLVGLFG DNENQSTVKT NSVFNMSLDQ
 SVVELYTDTA FSWSVGARAA LWECCATLG ASFQYAQSKP KVEELNVLCN AAFTINKPK
 GYVGQEFPLA LIAGTDAATG TKDASIDYHE WQASLALSYR LNMFTPYIGV KWSRASFDAD
 10 TIRIAQPKSA TAIFDITTLN PTIAGAGDVK ASAEGQLGDT MQIVSLQLNK MKSRKSCGIA
 VGTITVDADK YAVTVETRLI DERAHVNAQ FRF (SEQ ID NO: 2)

The native signal peptide sequence corresponds to residues 1-22 in the amino acid sequence of MOMP of *C. trachomatis* serovar E (SEQ ID NO: 2) and is shown underlined. Residues 23-393 of SEQ ID NO: 2 correspond to the mature form of full-length native MOMP polypeptide of serovar E of *C. trachomatis* which lacks the native signal peptide sequence. VD1-VD4 sequences of the MOMP polypeptide of serovar E of *C. trachomatis* are shown in bold and underlined text above.

A native *Chlamydia sp.* MOMP polypeptide may comprise the sequence of:

- (i) a native MOMP polypeptide of serovar D of *C. trachomatis* (SEQ ID NO: 1) or its mature form (residues 23-393 of SEQ ID NO: 1);
- 20 (ii) a native MOMP polypeptide of serovar F of *C. trachomatis*, (SEQ ID NO: 3), or its mature form (residues 23-395 of SEQ ID NO: 3); or
- (iii) a native MOMP polypeptide of serovar G of *C. trachomatis* (SEQ ID NO: 4) or its mature form (residues 23-395 of SEQ ID NO: 4).

A native *Chlamydia sp.* MOMP polypeptide comprises five conserved domains separated by four variable surface exposed domains (VD1, VD2, VD3 and VD4). For example, in the native MOMP polypeptide of *C. trachomatis* serovar E, VD1 (SEQ ID NO: 9) corresponds to amino acid residues 86-105 of SEQ ID NO: 2, VD2 (SEQ ID NO: 10) corresponds to amino acid residues 161-181 of SEQ ID NO: 2, VD3 (SEQ ID NO: 11) corresponds to amino acid residues 245-260 of SEQ ID NO: 2, and VD4 (SEQ ID NO: 12) corresponds to amino acid residues 309-337 of SEQ ID NO: 2. Conserved domains correspond to amino acid residues 23-85 (first conserved domain); 106-160 (second conserved domain); 182-244 (third conserved domain), 261-308 (fourth conserved domain) and 338-393 (fifth conserved domain). For serovar

E, either VD4 (SEQ ID NO: 12) corresponds to positions 309-337 in SEQ ID NO: 2 and the fifth conserved domain corresponds to positions 338-393 in SEQ ID NO: 2 or VD4 (SEQ ID NO: 883) corresponds to positions 309 - 338 in SEQ ID NO: 2 and the fifth conserved domain corresponds to positions 339 - 393 in SEQ ID NO: 2.

- 5 For serovars D, F and G of *C. trachomatis*, the residues corresponding to VD1, VD2, VD3 and VD4 and the five conserved domains are set out in Table 1. For serovar D, either VD4 (SEQ ID NO: 8) corresponds to positions 309-337 in SEQ ID NO: 1 and the fifth conserved domain corresponds to positions 338-393 in SEQ ID NO: 1 or VD4 (SEQ ID NO: 882) corresponds to positions 309-338 in SEQ ID NO: 1 and the fifth conserved domain corresponds to positions 339-393 in SEQ ID NO: 1. For serovar F, either VD4 (SEQ ID
- 10 NO: 16) corresponds to positions 310-339 in SEQ ID NO: 3 and the fifth conserved domain corresponds to positions 340-395 in SEQ ID NO: 3 or VD4 (SEQ ID NO: 884) corresponds to positions 310-340 in SEQ ID NO: 3 and the fifth conserved domain corresponds to positions 341-395 in SEQ ID NO: 3. For serovar G, either VD4 (SEQ ID NO: 20) corresponds to positions 310-339 in SEQ ID NO: 4 and the fifth conserved domain corresponds to positions 340-395 in SEQ ID NO: 4 or VD4 (SEQ ID NO: 885) corresponds to positions 310-340 in SEQ ID NO: 4 and the fifth conserved domain corresponds to positions 341-395 in
- 15 SEQ ID NO: 4.

Table 1 - VD1-VD4 and the first, second, third, fourth and fifth conserved domain in a native MOMP polypeptide of *C. trachomatis* serovars, D-G.

Serovar	Domain		Positions in SEQ ID NO:	SEQ ID NOS
D	First conserved domain		23-85 in SEQ ID NO: 1	
	VD1		86-105 in SEQ ID NO: 1	5
	Second conserved domain		106-160 in SEQ ID NO: 1	
	VD2		161-181 in SEQ ID NO: 1	6
	Third conserved domain		182-244 in SEQ ID NO: 1	
	VD3		245-260 in SEQ ID NO: 1	7
	Fourth conserved domain		261-308 in SEQ ID NO: 1	
	Either	VD4	309-337 in SEQ ID NO: 1	8
		Fifth conserved domain	338-393 in SEQ ID NO: 1	
	Or	VD4	309-338 in SEQ ID NO: 1	882
		Fifth conserved domain	339-393 in SEQ ID NO: 1	
E	First conserved domain		23-85 in SEQ ID NO: 2	
	VD1		86-105 in SEQ ID NO: 2	9
	Second conserved domain		106-160 in SEQ ID NO: 2	
	VD2		161-181 in SEQ ID NO: 2	10
	Third conserved domain		182-244 in SEQ ID NO: 2	
	VD3		245-260 in SEQ ID NO: 2	11

	Fourth conserved domain		261-308 in SEQ ID NO: 2	
	Either	VD4	309-337 in SEQ ID NO: 2	12
		Fifth conserved domain	338-393 in SEQ ID NO: 2	
	Or	VD4	309-338 in SEQ ID NO: 2	883
		Fifth conserved domain	339-393 in SEQ ID NO: 2	
F	First conserved domain		23-85 in SEQ ID NO: 3	
	VD1		86-106 in SEQ ID NO: 3	13
	Second conserved domain		107-161 in SEQ ID NO: 3	
	VD2		162-182 in SEQ ID NO: 3	14
	Third conserved domain		183-245 in SEQ ID NO: 3	
	VD3		246-261 in SEQ ID NO: 3	15
	Fourth conserved domain		262-309 in SEQ ID NO: 3	
	Either	VD4	310-339 in SEQ ID NO: 3	16
		Fifth conserved domain	340-395 in SEQ ID NO: 3	
	Or	VD4	310-340 in SEQ ID NO: 3	884
		Fifth conserved domain	341-395 in SEQ ID NO: 3	
G	First conserved domain		23-85 in SEQ ID NO: 4	
	VD1		86-106 in SEQ ID NO: 4	17
	Second conserved domain		107-161 in SEQ ID NO: 4	
	VD2		162-182 in SEQ ID NO: 4	18
	Third conserved domain		183-245 in SEQ ID NO: 4	
	VD3		246-261 in SEQ ID NO: 4	19
	Fourth conserved domain		262-309 in SEQ ID NO: 4	
	Either	VD4	310-339 in SEQ ID NO: 4	20
		Fifth conserved domain	340-395 in SEQ ID NO: 4	
	Or	VD4	310-340 in SEQ ID NO: 4	885
		Fifth conserved domain	341-395 in SEQ ID NO: 4	

Native *C. trachomatis* MOMP polypeptides of serovars A-C, H-K, or L1-L3, or other native *Chlamydia sp.* MOMP polypeptides comprise VD1-VD4 and conserved domains at positions corresponding to the residue numbering set out for the native MOMP polypeptides of serovars D-G of *C. trachomatis*, e.g., serovar E.

- 5 The sequences of other native *Chlamydia sp.* MOMP polypeptides, including those of *C. trachomatis* MOMP polypeptides of serovars A-C, H-K, or L1-L3 are well known and available from public databases. For example, amino acid sequences of native *Chlamydia sp.* MOMP polypeptides are available on the Uniprot (<https://www.uniprot.org/>) and NCBI (<https://www.ncbi.nlm.nih.gov/>) databases. Nucleic acid sequences encoding native *Chlamydia sp.* MOMP polypeptides may be obtained from the NCBI
- 10 (<https://www.ncbi.nlm.nih.gov/>) database. The positions of VDs and conserved domains within other native *Chlamydia sp.* MOMP polypeptides are well known and in any event can be determined by aligning

sequences of e.g., *C. trachomatis* serovars D, E, F, and G with sequences of other native *Chlamydia sp.* MOMP polypeptides and looking for regions of high sequence conservation to identify e.g., conserved domains.

Modified MOMP polypeptides

5 The inventors have generated modified MOMP polypeptides comprising non-native loop sequences instead of native *Chlamydia sp.* MOMP VD sequences. The inventors have recognised that such modified MOMP polypeptides can be used to elicit a protective immune response against *Chlamydia sp.* infection. In particular, the inventors have demonstrated that such modified MOMP polypeptides induce a T cell response, including CD4+ T cells such as IFN γ -producing CD4+ T cells. Notably, IFN- γ producing CD4+
10 T cells are thought to be important in protecting against *Chlamydia sp.* infections. Elimination of native VD sequences may therefore reduce B cell-mediated (e.g., antibody) responses (e.g., to B cell epitopes in VDs), while prioritising T cell responses to MOMP, e.g., to sequences in the conserved domains which are conserved between serovars. The inventors have shown that modified MOMP polypeptides can elicit a cross-serovar T cell immune response, i.e., a T cell immune response that is cross-reactive against two or
15 more serovars of *Chlamydia sp.*, e.g., serovars D-G and J of *C. trachomatis*.

The inventors have therefore demonstrated that these modified MOMP polypeptides are suitable vaccine candidates that may be used either as stand-alone antigens or in combination with other *Chlamydia sp.* antigens that can elicit B cell-mediated (e.g., antibody) responses and/or T cell-mediated responses in a subject, such as one or more chimeric MOMP VD polypeptides provided herein and/or one or more
20 *Chlamydia sp.* CT443, CT584, CT600, or CT812 polypeptides provided herein, in order to promote long-lasting memory T cell responses and protective immunity against infection. A modified MOMP polypeptide as described herein may be delivered by a nucleic acid comprising a nucleotide sequence encoding the modified MOMP polypeptide.

Accordingly, in one aspect, the invention provides a nucleic acid comprising a nucleotide sequence
25 encoding a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences. In a further aspect, the invention provides a modified MOMP polypeptide having an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop
30 sequence between the conserved domain sequences.

In a preferred embodiment, the modified MOMP polypeptide does not comprise a native *Chlamydia sp.* MOMP variable domain between the two or more conserved domain sequences. Thus, in a modified MOMP polypeptide, a non-native loop sequence may replace a native *Chlamydia sp.* MOMP polypeptide VD sequence. A modified MOMP polypeptide may therefore omit a native *Chlamydia sp.* MOMP VD

sequence between the two or more conserved domain sequences and include a non-native loop sequence between the two or more conserved domain sequences.

Replacing native *Chlamydia sp.* MOMP VD sequences with non-native loops removes B cell epitopes (e.g., antibody epitopes) found in the native *Chlamydia sp.* MOMP VDs. Removal of these B cell epitopes may reduce B cell-mediated responses to the antigen in a subject (e.g., to B cell epitopes in native MOMP VDs). This may advantageously increase T cell responses to MOMP in a subject, e.g., to sequences in the MOMP conserved domains which share a high degree of identity between MOMP serovars. The modified MOMP polypeptides of the invention may therefore elicit a cross-serovar T cell immune response in a subject, i.e. a T cell immune response that is cross-reactive against two or more serovars of *Chlamydia sp.* (e.g., cross-reactive against two or more serovars (e.g., serovars D-G and J) of *C. trachomatis*). Thus, the modified MOMP polypeptides of the invention may be capable of enhancing T cell-mediated responses (e.g., relative to a corresponding native MOMP polypeptide). The modified MOMP polypeptides of the invention may be capable of enhancing a CD4+ T cell-mediated response (e.g., a IFN γ + CD4+ T cell response such as IFN γ + IL2+ TNF α + CD4+ T cell response) e.g., relative to a corresponding native MOMP polypeptide. The modified MOMP polypeptides of the invention may be capable of inducing a T cell response (e.g., an antigen specific T cell response) in a subject. The T cell response may be immunodominant. Thus, the modified MOMP polypeptide of the invention may be capable of eliciting a T cell population responsive to a native *Chlamydia sp.* MOMP polypeptide in a subject. In some embodiments, the T cell response is a CD4+ T cell response (e.g., IFN γ + CD4+ T cell response). In some embodiments, the T cell response is a IFN γ + IL2+ TNF α + CD4+ T cell response.

“Non-native loop sequences” refer to sequences that are not native to any *Chlamydia sp.* MOMP VD (VD1, VD2, VD3 or VD4) of any serovar. In some embodiments, non-native loop sequences are no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10% or no more than 5% identical to any native *Chlamydia sp.* MOMP VD (VD1, VD2, VD3 or VD4) sequences of any serovar. In some embodiments, non-native loop sequences are no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10% or no more than 5% identical to MOMP VD sequences (VD1, VD2, VD3 or VD4) of serovar E of *C. trachomatis* (SEQ ID Nos: 9-12).

Non-native loop sequences may allow the conserved domain sequences in the modified MOMP polypeptide to form a beta-barrel structure, e.g., to maintain a beta-barrel structure of the conserved domains of a native *Chlamydia sp.* MOMP polypeptide. Thus, in the modified MOMP polypeptides of the invention, the two or more conserved domain sequences are linked by a non-native loop sequence such that the conserved domain sequences form a beta-barrel structure (e.g., made up of anti-parallel beta strands). The beta-barrel structure may be as predicted *in silico* (e.g., using Alphafold2 (Deepmind) software).

In silico modelling may be used to create a 3D structure of a native MOMP protein, which comprises a beta barrel with VD1-VD4 sequences as loops linking individual beta strands. The VD1-VD4 loops are located on the same side of the barrel structure (surface exposed in a native MOMP polypeptide). Non-native loop sequences may be mapped onto VD loops in an *in silico* model and suitable non-native loop sequences may be determined by the skilled person. Suitable non-native loop sequences include peptide sequences that can cover the distance between the ends of the conserved domains flanking a native VD, e.g., as modelled *in silico*. The distance may correspond to the distance in e.g., angstrom as determined by *in silico* 3D model between the last conserved domain residue before the VD sequence to the first conserved domain residue after the VD sequence.

Non-native loop sequences may exclude sequence motifs that are found in a subject (e.g., human) proteome, e.g., sequence motifs of 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more or 15 or more amino acids in length. Excluded subject proteome (e.g., human proteome) sequence motifs are typically 8 or more amino acids in length. This helps minimise unwanted cross-reactivity due to homology between the antigen and self protein.

A non-native loop sequence may be between 3 and 30 amino acids in length, e.g., between 4 and 20 amino acids in length. In some embodiments, a non-native loop sequence replacing VD1 may have a sequence according to SEQ ID NO: 462 or 466 (e.g., SEQ ID NO: 462). In some embodiments, a non-native loop sequence replacing VD2 may have a sequence according to SEQ ID NO: 463 or 467 (e.g., SEQ ID NO: 463). In some embodiments, a non-native loop sequence replacing VD3 may have a sequence according to SEQ ID NOs 464 or 468 (e.g., SEQ ID NO: 464). In some embodiments, a non-native loop sequence replacing VD4 may have a sequence according to SEQ ID NO: 465 or 469 (e.g., SEQ ID NO: 465). Typically, a modified MOMP polypeptide may comprise four non-native loop sequences according to SEQ ID NOs 462-465 in place of VD1, VD2, VD3 and VD4, respectively. A modified MOMP polypeptide may alternatively comprise four non-native loop sequences according to SEQ ID NOs 466-469 in place of VD1, VD2, VD3 and VD4, respectively.

A “conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide” refers to a sequence of a conserved domain of a MOMP polypeptide of a *Chlamydia* species of any serovar, or a variant thereof. Variants include sequences having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity (preferably at least 95% identity) to a conserved domain of a MOMP polypeptide of a *Chlamydia sp.* of any serovar. In some embodiment, the conserved domain sequences of the modified MOMP polypeptide is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical (at least 95% identical) a conserved domain of a native MOMP polypeptide of a *Chlamydia sp.* of any serovar (e.g., a conserved domain as defined in Table 1). Variants also include truncated forms of native conserved domains of a

MOMP polypeptide of a *Chlamydia sp.*, for example wherein a conserved domain sequence in a modified MOMP polypeptide lacks up to 3, 5, 8, 10, 15, 20, 25 or 30 (e.g., up to 3 or 5, e.g., up to 3) amino acids of a native *Chlamydia sp.* MOMP conserved domain sequence. In some embodiments, the conserved domain sequences of the modified MOMP polypeptide lack up to 3 or 5 amino acids of a native *Chlamydia sp.* MOMP conserved domain sequence. The conserved domain boundaries for *C. trachomatis* serovars D, E, F and G are set out in Table 1.

In some embodiments, the modified MOMP polypeptide comprises two, three, four or five conserved domains of a native *Chlamydia sp.* MOMP polypeptide, or variants thereof. In some embodiments, a modified MOMP polypeptide comprises five conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide, or variants thereof. In some embodiments, the modified MOMP polypeptide comprises all five full-length conserved domains of a native *Chlamydia sp.* MOMP polypeptide. The conserved domains of the modified MOMP polypeptide may collectively have at least 95% sequence identity to the conserved domains of a native MOMP polypeptide (e.g., serovar E MOMP). In some embodiments, the conserved domain sequences of the modified MOMP polypeptide collectively have at least 90% or at least 95% sequence (e.g., at least 95%) identity to the conserved domains of a native MOMP polypeptide (e.g., the conserved domains as defined in Table 1).

Conserved domains of a modified MOMP polypeptide may comprise conserved domain sequences of MOMP from a serovar of *C. trachomatis* (e.g., serovars D-K, such as serovars D-G). In some embodiments, conserved domains of a modified MOMP polypeptide may comprise conserved domain sequences of MOMP from serovar E of *C. trachomatis*.

A modified MOMP polypeptide may comprise a non-native loop sequence between each of the conserved domain sequences. Thus, a modified MOMP polypeptide may comprise one, two, three or four non-native loop sequences. The one or more non-native loop sequence(s) may replace one, two, three or four of the corresponding native VD loops. Native VDs may be retained between any remaining conserved domains. The modified MOMP polypeptide may have five conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide. In some embodiments, the modified MOMP polypeptide does not comprise any native *Chlamydia sp.* MOMP VDs between any of the conserved domain sequences. In some embodiments, the modified MOMP polypeptide comprises four non-native loop sequences and does not comprise any native *Chlamydia sp.* MOMP VDs between the conserved domain sequences. In some embodiments, the modified MOMP polypeptide does not comprise any native *Chlamydia sp.* MOMP VDs (or fragments of at least 5, 6, or 7 consecutive amino acids thereof).

In some embodiments, the modified MOMP polypeptide comprises (1) all five conserved domain sequences of a *Chlamydia sp.* MOMP polypeptide of *C. trachomatis* of any serovar, and (2) four non-native loop sequences, wherein a non-native loop sequence is located between each of the conserved domain sequences, and wherein the modified MOMP polypeptide does not comprise any native *Chlamydia sp.* MOMP variable

domains between any of the conserved domain sequences, further wherein the non-native loop sequences are between 3 and 30 amino acids in length and no more than 40% identical to any native *Chlamydia sp.* MOMP VD (VD1, VD2, VD3 or VD4) sequences of any serovar.

5 A modified MOMP polypeptide may comprise an amino acid sequence (e.g., from N terminus to C terminus) according to formula:

C1 – L1 – C2 (Formula I),

wherein C1 and C2 are two conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and L1 is a non-native loop sequence.

10 In some embodiments, the modified MOMP polypeptide comprises an amino acid sequence (e.g., from N terminus to C terminus) according to formula:

C1 – L1 – C2 – L2 – C3 (Formula II),

wherein C1, C2 and C3 are three conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and L1 and L2 are non-native loop sequences.

15 In some embodiments, a modified MOMP polypeptide comprises an amino acid sequence (e.g., from N terminus to C terminus) according to formula:

C1 – L1 – C2 – L2 – C3 – L3 – C4 (Formula III),

wherein C1, C2, C3 and C4 are four conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and L1, L2 and L3 are non-native loop sequences.

20 In some embodiments, a modified MOMP polypeptide comprises an amino acid sequence (e.g., from N terminus to C terminus) according to formula:

C1 – L1 – C2 – L2 – C3 – L3 – C4 – L4 – C5 (Formula IV)

wherein C1, C2, C3, C4 and C5 are five conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and L1, L2, L3 and L4 are non-native loop sequences.

The conserved domain sequences and non-native loop sequences may be as described herein.

25 In some embodiments, C1-C5 correspond to the first, second, third, fourth and fifth conserved domains of a native *Chlamydia trachomatis* MOMP polypeptide, respectively (e.g., as defined in Table 1).

Accordingly, in some embodiments, a nucleic acid of the invention comprises a nucleotide sequence encoding a modified MOMP polypeptide which comprises or consists of the sequence set out in Formula I, II, III or IV, preferably IV.

In some embodiments, the modified MOMP polypeptide comprises a sequence according to any one of SEQ ID NO: 486-489 (e.g., SEQ ID NO: 486) or a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, a modified MOMP polypeptide comprises a sequence having at least 90% identity to SEQ ID NO 486-489 (e.g., SEQ ID NO: 486). In some embodiments, a modified MOMP polypeptide comprises a sequence having at least 95% identity to SEQ ID NO 486-489 (e.g., SEQ ID NO: 486).

In some embodiments, the nucleic acid comprising a nucleotide sequence encoding a modified MOMP polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 551-566 (e.g., SEQ ID NO: 551) or a sequence that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% (e.g., at least 75%) identity thereto. Typically, the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% (e.g., at least 75%) identity thereto. In some embodiments, the nucleic acid comprises a nucleotide sequence encoding a secretion signal peptide sequence as described herein, e.g., a secretion signal peptide sequence according to SEQ ID NO: 187.

In one embodiment, the nucleic acid of the invention is a mRNA comprising or consisting of (e.g., consisting of) the following structural elements:

(1) a 5' cap;

(2) a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;

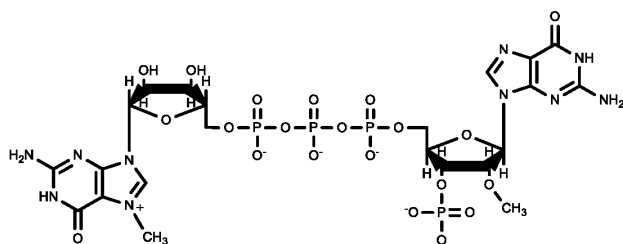
(3) a protein coding region having the nucleic acid sequence according to SEQ ID NO: 213 optionally followed by a stop codon (e.g., TGA);

(4) a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and

(5) a polyA tail.

In one embodiment, the nucleic acid of the invention is a mRNA comprising or consisting of (e.g., consisting of) the following structural elements:

(1) a 5' cap with the following structure:



(2) a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;

(3) a protein coding region having the nucleic acid sequence according to SEQ ID NO: 213;

(4) a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and

5 (5) a polyA tail.

In some embodiments, the 3' end of (1) bonds directly to the 5' end of (2) via a 3' to 5' phosphodiester linkage; the 3' end of (2) bonds directly to the 5' end of (3) via a 3' to 5' phosphodiester linkage; the 3' end of (3) bonds directly to the 5' end of (4) via a 3' to 5' phosphodiester linkage; and the 3' end of (4) bonds directly to the 5' end of (5) via a 3' to 5' phosphodiester linkage. In some embodiments, the mRNA is
10 chemically modified and the chemical modification comprises N1-methylpseudouridine in place of every uridine. The mRNA may be encapsulated in a LNP.

Chimeric MOMP VD polypeptides

The inventors have demonstrated that immunisation with chimeric *Chlamydia sp.* MOMP VD polypeptides comprising VD sequences of different *C. trachomatis* serovars elicited potent antibody responses. In
15 particular, the inventors have shown that mRNAs encoding chimeric MOMP VD polypeptides which combined VD sequences of serovars that show lower levels of VD sequence identity with one another relative to other serovars successfully elicited potent antibody (IgG) responses. Chimeric MOMP VD polypeptides containing VD domains of which only one is from a particular serovar were able to elicit robust IgG responses against that serovar. IgG antibody response levels obtained using mRNAs encoding
20 chimeric MOMP VD polypeptides were higher than those induced by immunisation with a mRNA encoding a polypeptide combining four VD4 sequences of serovars D, E, F and G (which was based on the polypeptide described in Anja W. Olsen, et al., Protection Against Chlamydia trachomatis Infection and Upper Genital Tract Pathological Changes by Vaccine-Promoted Neutralizing Antibodies Directed to the VD4 of the Major Outer Membrane Protein, The Journal of Infectious Diseases, Volume 212, Issue 6, 2015,
25 Pages 978–989). Furthermore, IgG antibody response levels obtained using mRNAs encoding chimeric MOMP VD polypeptides were higher than those induced by immunisation with a mRNA encoding a native MOMP protein. The inventors have also demonstrated that immunisation with chimeric *Chlamydia sp.* MOMP VD polypeptides of the invention elicited a potent antibody (e.g., IgG) response against *C. trachomatis* elementary bodies (EBs). The elicited antibodies were cross-reactive against EBs of different

serovars of *C. trachomatis*. The inventors have also demonstrated that a *Chlamydia* sp. MOMP VD polypeptide of the invention elicited a T cell response, including CD4+ and CD8+ T cells, such as IFN γ -producing CD4+ and CD8+ T cells. The inventors have demonstrated that the chimeric MOMP VD polypeptides of the invention are suitable vaccine candidates.

5 Thus, in another aspect, the invention provides a nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia* sp. MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia* sp. MOMP VD sequences of different serovars of the *Chlamydia* sp. In a further aspect, the invention provides a chimeric *Chlamydia* sp. MOMP VD polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino
10 acid sequence comprising two or more *Chlamydia* sp. MOMP VD sequences of different serovars of the *Chlamydia* sp. Native MOMP VD sequences are highly variable across *Chlamydia* sp. serovars and comprise epitopes that elicit a B cell (i.e., antibody) immune response. A chimeric MOMP VD polypeptide of the present invention may elicit a B cell (i.e., antibody) response, e.g., an antigen-specific antibody response, in a subject. The antibody response may be an IgG response. The elicited antibodies may bind *C.*
15 *trachomatis* elementary bodies. The terms “chimeric MOMP VD construct”, “chimeric *Chlamydia* sp. MOMP VD polypeptide” and “chimeric MOMP VD polypeptide” are used herein interchangeably.

Thus, a chimeric MOMP VD polypeptide of the invention comprising two or more MOMP VD sequences of different *Chlamydia* sp. serovars may elicit a B cell (i.e. antibody) response against more than one serovar of *C. trachomatis*, e.g., a cross-serovar response. Thus, the chimeric VD polypeptide of the present
20 invention may elicit a B cell (antibody) response against two or more serovars of *Chlamydia* sp. The response may be a protective antibody response.

The inventors compared MOMP VD sequences of *C. trachomatis* and found different levels of sequence variation across serovars. MOMP VD sequences of some of the serovars were identical or had a high level of sequence identity (e.g., at least 70%) whereas for other serovars there was a greater degree of sequence
25 variation. For example, VD1 sequences of serovars D and E have a high level of sequence identity and VD1 sequences of serovars F and G are identical. However, there is a much greater level of variation between VD1 sequences of (i) serovar D or E and (ii) serovar F or G. VD2 sequences of serovars D and E have a high level of sequence identity, and likewise VD2 sequences of serovars F and G have a high level of sequence identity. However, there is a much greater level of variation between VD2 sequences of (i) serovar
30 D or E and (ii) serovar F or G. VD3 sequences of serovars D and F are identical, and VD3 sequences of serovars F and G have a high level of sequence identity. VD3 sequence of serovar E has a lower percentage of sequence identity compared to VD3 sequences of serovars D / F than compared to serovar G. However, the inventors have found that VD3 sequence of serovar E contains amino acid motifs that are homologous with sequences found in human proteome. For VD4, the sequences of serovars D and E have a high level
35 of sequence identity, and likewise the sequences of serovars F and G have a high level of sequence identity.

However, there is a much greater level of sequence variation between VD4 sequences of (i) serovar D or E and (ii) serovar F or G.

5 A chimeric MOMP VD polypeptide of the invention may therefore comprise two MOMP VD1 sequences of different serovars of a *Chlamydia sp.*, wherein the two VD1 sequences have a lower sequence identity with one another than with VD1 sequences of other serovars of the *Chlamydia sp.* In some embodiments, the two MOMP VD1 sequences of different serovars of a *Chlamydia sp.* in the chimeric MOMP VD polypeptide are no more than 70%, 69%, 68%, 67%, 66%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25% identical (e.g., no more than 50% identical) to each other. The serovars may be selected from *C. trachomatis* serovars D-K, e.g., D, E, F, and G.

10 A chimeric MOMP VD polypeptide of the invention may comprise two MOMP VD2 sequences of different serovars of a *Chlamydia sp.*, wherein the two VD2 sequences have a lower sequence identity with one another than with VD2 sequences of other serovars of the *Chlamydia sp.* In some embodiments, the two MOMP VD2 sequences of different serovars of a *Chlamydia sp.* in the chimeric MOMP VD polypeptide are no more than 80%, 79%, 78%, 77%, 76%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25% identical (e.g., no more than 50%) to each other. The serovars may be selected from *C. trachomatis* serovars D-K, e.g., D, E, F, and G.

A chimeric MOMP VD polypeptide of the invention may comprise two MOMP VD3 sequences of different serovars of a *Chlamydia sp.*, wherein the two VD3 sequences have a lower sequence identity with one another than with VD3 sequences of other serovars of the *Chlamydia sp.* In some embodiments, the two MOMP VD3 sequences of different serovars of a *Chlamydia sp.* in the chimeric MOMP VD polypeptide are no more than 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25% identical (e.g., no more than 70%) to each other. In other embodiments, the chimeric MOMP comprises a single MOMP VD3 domain. The serovars may be selected from *C. trachomatis* serovars D-K, e.g., D, E, F, and G.

25 A chimeric MOMP VD polypeptide of the invention may comprise two MOMP VD4 sequences of different serovars of a *Chlamydia sp.*, wherein the two VD4 sequences have a lower sequence identity with one another than with VD4 sequences of other serovars of the *Chlamydia sp.* In some embodiments, the two MOMP VD4 sequences of different serovars of the *Chlamydia sp.* in a chimeric MOMP VD polypeptide may be no more than 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25% identical (e.g., no more than 60% identical) to each other. The serovars may be selected from *C. trachomatis* serovars D-K, e.g., D, E, F, and G.

In preferred embodiments, MOMP VD sequences in the chimeric MOMP VD polypeptide exclude sequence motifs that are found in a subject (e.g., human) proteome, e.g., sequence motifs of 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more or 15 or more (e.g., 8 or more) amino

acids in length. Excluded subject proteome (e.g., human proteome) sequence motifs are typically 8 or more amino acids in length. As explained above, this helps minimise unwanted cross-reactivity.

5 In preferred embodiments, the chimeric MOMP VD polypeptide comprises conserved domain sequence portions of a native *Chlamydia sp.* MOMP polypeptide flanking each of the two or more MOMP VD sequences.

The chimeric MOMP VD polypeptide may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 MOMP VD sequences. In some embodiments, the chimeric MOMP VD polypeptide comprises seven MOMP VD sequences.

The *Chlamydia sp.* may be *C. trachomatis* and the serovars may be D-K.

10 The chimeric MOMP VD polypeptide may comprise MOMP VDs of two, three, four, five, six, seven or eight different serovars, e.g., serovars D-K of *C. trachomatis*. In some embodiments, the chimeric MOMP VD polypeptide comprises MOMP VDs of four different serovars, e.g., serovars D-G of *C. trachomatis*.

15 The chimeric MOMP VD polypeptide may comprise (i) two MOMP VD1 sequences of different serovars of the *Chlamydia sp.*; and/or (ii) two MOMP VD2 sequences of different serovars of the *Chlamydia sp.*; and/or (iii) one MOMP VD3 sequence; and/or (iv) two MOMP VD4 sequences of different serovars of the *Chlamydia sp.* In other embodiments, there are two MOMP VD3 sequences of different serovars of the *Chlamydia sp.* The different serovars may be selected from serovars D, E, F or G of *C. trachomatis*.

20 A chimeric MOMP VD polypeptide of the invention may therefore comprise one MOMP VD sequence of serovar D or E of *C. trachomatis* and one MOMP VD sequence of serovar F or G of *C. trachomatis*. In some embodiments, the chimeric MOMP VD polypeptide comprises a MOMP VD1 sequence of serovar D or E of *C. trachomatis* and a MOMP VD1 sequence of serovar F or G of *C. trachomatis* (for example, a MOMP VD1 sequence of serovar E of *C. trachomatis* and a MOMP VD1 sequence of serovar G of *C. trachomatis*). In some embodiments, the chimeric MOMP VD polypeptide comprises a MOMP VD2 sequence of serovar D or E of *C. trachomatis* and a MOMP VD2 sequence of serovar F or G of *C. trachomatis* (for example, a MOMP VD2 sequence of serovar D of *C. trachomatis* and a MOMP VD2 sequence of serovar G of *C. trachomatis*). In some embodiments, the chimeric MOMP VD polypeptide comprises a MOMP VD3 sequence of serovar D, F or G of *C. trachomatis* (for example, serovar F). In some embodiments, the chimeric MOMP VD polypeptide comprises a MOMP VD4 sequence of serovar D or E of *C. trachomatis* and a MOMP VD4 sequence of serovar F or G of *C. trachomatis* (for example, a MOMP VD4 sequence of serovar D of *C. trachomatis* and a MOMP VD4 sequence of serovar F of *C. trachomatis*).

A chimeric MOMP VD polypeptide of the invention may comprise two, three or four MOMP VD sequences of serovars D or E of *C. trachomatis* and two, three or four MOMP VD sequences of serovars F or G of *C.*

trachomatis. In some embodiments, the chimeric MOMP VD polypeptide comprises one MOMP VD sequence of serovar D or E of *C. trachomatis* and one MOMP VD sequence of serovar F or G of *C. trachomatis* for each one of MOMP VD1, VD2, VD3, and VD4. In preferred embodiments, the chimeric MOMP VD polypeptide comprises only one MOMP VD3 sequence, which corresponds to VD3 of serovar D, F or G of *C. Trachomatis* (e.g., serovar F).

A chimeric MOMP VD polypeptide may comprise at least one (e.g., one, two, three or four) of (i)-(iv): (i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, or a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F; and/or (ii) a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, or a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G; and/or (iii) a MOMP VD3 sequence from serovar G, or a MOMP VD3 sequence from serovar F; and/or (iv) a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G, or a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F, wherein the serovars D, E, F or G are of *C. trachomatis*. In some embodiments, the chimeric MOMP VD polypeptide comprises four of (i)-(iv). In some embodiments, the chimeric MOMP VD polypeptide comprises (i) a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar G; and (ii) a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G; and (iii) a MOMP VD3 sequence from serovar F; and (iv) a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F, wherein the serovars D, E, F or G are of *C. trachomatis*. The MOMP VD1 sequence from serovar F of *C. trachomatis* is the same as the MOMP VD1 sequence from serovar G of *C. trachomatis*.

In some embodiments, the chimeric MOMP VD polypeptide comprises: (i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, a MOMP VD3 sequence from serovar G, a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G; or (ii) a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G, a MOMP VD3 sequence from serovar F, a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F, wherein the serovars D, E, F or G are of *C. trachomatis*. In some embodiments, the chimeric MOMP VD polypeptide comprises MOMP VD sequences as in (i). Typically, the chimeric MOMP VD polypeptide comprises MOMP VD sequences as in (ii).

Typically, the chimeric MOMP VD polypeptide comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to an N-glycosylation site in a native *Chlamydia sp.* MOMP polypeptide, e.g., in one or more MOMP VD domains.

A chimeric MOMP VD polypeptide as described herein may comprise conserved domain sequence portions that are portions of conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide flanking

the VD in its native *Chlamydia sp.* MOMP polypeptide. The conserved domain sequences flanking the VD in its native *Chlamydia sp.* MOMP polypeptide may be immediately adjacent to the VD sequence.

Each conserved domain sequence portion in a chimeric MOMP VD polypeptide may comprise between 3 and 30 amino acid residues (e.g., between 4 and 20) of a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, wherein the between 3 and 30 (e.g., between 4 and 20) amino acid residues are immediately adjacent to the VD sequence in its native *Chlamydia sp.* MOMP polypeptide.

A chimeric MOMP VD polypeptide as described herein may comprise an amino acid sequence according to the following formula:

$(Ax - VDx - Bx)y - (Aw - VDw - Bw)z$, wherein

10 VDx comprises a VD sequence of VD1, VD2, VD3 or VD4 of a native *C. trachomatis* serovar y MOMP polypeptide;

Ax comprises between 3 and 30 (e.g., between 4 and 20) amino acid residues that immediately precede VDx in a native *Chlamydia sp.* MOMP polypeptide sequence of serovar y;

15 Bx comprises between 3 and 30 (e.g., between 4 and 20) amino acid residues that immediately follow VDx in a native *Chlamydia sp.* MOMP polypeptide sequence of serovar y;

VDw comprises a VD sequence of VD1, VD2, VD3 or VD4 of a native *C. trachomatis* serovar z MOMP polypeptide;

Aw comprises between 3 and 30 (e.g., between 4 and 20) amino acid residues that immediately precede VDw in a native *Chlamydia sp.* MOMP polypeptide sequence of serovar z;

20 Bw comprises between 3 and 30 (e.g., between 4 and 20) amino acid residues that immediately follow VDw in a native *Chlamydia sp.* MOMP polypeptide sequence of serovar z,

x and w are independently selected from 1-4 and indicate the MOMP VD selected from VD1, VD2, VD3 or VD4; and

y and z are serovars of *C. trachomatis* (e.g., selected from serovars D, E, F, or G).

25 In some embodiments, the chimeric MOMP VD polypeptide comprises a sequence according to any one of SEQ ID NO: 490-505 (e.g., SEQ ID NO: 495 or 503 (e.g., SEQ ID NO: 503)) or a sequence that has at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some
30 embodiments, the chimeric MOMP VD polypeptide comprises a sequence having at least 90% identity to SEQ ID NO 490-505 (e.g., SEQ ID NO: 495 or 503 (e.g., SEQ ID NO: 503). In some embodiments, the

chimeric MOMP VD polypeptide comprises a sequence having at least 95% identity to any one of SEQ ID NO 490-505 (e.g., SEQ ID NO: 495 or 503 (e.g., SEQ ID NO: 503).

In some embodiments, a nucleic acid comprising a nucleotide sequence encoding the chimeric MOMP VD polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 567-630 (e.g., SEQ ID NO: 617) or a sequence that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, a nucleic acid comprising a nucleotide sequence encoding the chimeric MOMP VD polypeptide comprises a nucleotide sequence that is least 85% identical to any one of SEQ ID NO: 567-630 (e.g., SEQ ID NO: 617).

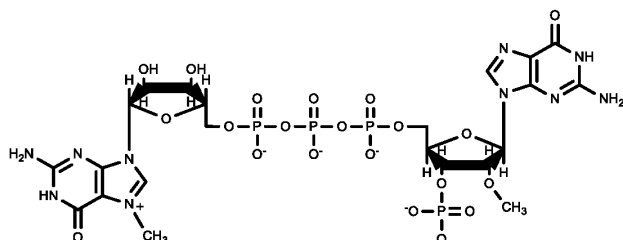
In some embodiments, the nucleic acid comprises a nucleotide sequence encoding a secretion signal peptide sequence as described herein, e.g., a secretion signal peptide sequence according to SEQ ID NO: 187. For example, the nucleic acid may comprise a sequence according to SEQ ID NO: 870.

In one embodiment, the nucleic acid is a mRNA comprising or consisting of (e.g., consisting of) the following structural elements:

- (1) a 5' cap;
- (2) a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- (3) a protein coding region having the nucleic acid sequence according to SEQ ID NO: 870 optionally followed by a stop codon (e.g., TGA);
- (4) a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- (5) a polyA tail.

In one embodiment, the nucleic acid is a mRNA comprising or consisting of (e.g., consisting of) the following structural elements:

- (1) a 5' cap with the following structure:



- (2) a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- (3) a protein coding region having the nucleic acid sequence according to SEQ ID NO: 870;

- (4) a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
(5) a polyA tail.

In some embodiments, the 3' end of (1) bonds directly to the 5' end of (2) via a 3' to 5' phosphodiester linkage; the 3' end of (2) bonds directly to the 5' end of (3) via a 3' to 5' phosphodiester linkage; the 3' end of (3) bonds directly to the 5' end of (4) via a 3' to 5' phosphodiester linkage; and the 3' end of (4) bonds directly to the 5' end of (5) via a 3' to 5' phosphodiester linkage. In some embodiments, the mRNA is chemically modified and the chemical modification comprises N1-methylpseudouridine in place of every uridine. The mRNA may be encapsulated in a LNP.

In alternative embodiments, a chimeric MOMP VD polypeptide comprises three MOMP VD4 sequences selected from (i) a MOMP VD4 sequence of serovar D of *C. trachomatis*; (ii) a MOMP VD4 sequence of serovar E of *C. trachomatis*; (iii) a MOMP VD4 sequence of serovar F of *C. trachomatis*; or (iv) a MOMP VD4 sequence of serovar G of *C. trachomatis*. In some embodiments, the chimeric MOMP VD polypeptide comprises all four VD4 domains (i)-(iv). In some embodiments, the chimeric MOMP VD polypeptide comprises a sequence according to any one of SEQ ID NOs: 506, 841, 842 or 843 or a sequence with at least 90% or at least 95% identity thereto.

Chlamydia sp. antigens other than MOMP

The inventors have identified *Chlamydia sp.* antigens other than MOMP ("non-MOMP antigens") which may be used in the present invention. Non-MOMP antigens or the nucleic acids encoding them may be provided or used in combination with MOMP antigens, including the MOMP antigens (and the nucleic acids encoding them) described herein. These non-MOMP antigens include *Chlamydia sp.* polypeptides CT443, CT584, CT600 and CT812. These antigens are highly abundant outer membrane proteins and are highly conserved across *Chlamydia sp.*, e.g., across serovars of *C. trachomatis*. The use of any one or more of these antigens may therefore provide a cross-serovar immune response, i.e., an immune response that is cross-reactive against two or more serovars of *Chlamydia sp.* (e.g., cross-reactive against two or more serovars of *C. trachomatis*). The inventors have demonstrated that *C. trachomatis* polypeptides CT443, CT584, CT600 and CT812 elicited a T cell response when delivered either as mRNAs expressing the relevant antigen or in the form of recombinant proteins. The inventors have also shown that at least mRNAs encoding *C. trachomatis* polypeptides CT443, CT584 or CT600 as well as recombinant CT443, CT584 or CT600 proteins induced robust antigen-specific antibody responses.

CT443

The inventors have demonstrated that both mRNA encoding *C. trachomatis* CT443 polypeptide and a recombinant *C. trachomatis* CT443 protein elicited IFN γ -producing T cells. Furthermore, *C. trachomatis* CT443 polypeptide elicited a robust IgG response when delivered either as a mRNA expressing the antigen

or in the form of a recombinant protein. *C. trachomatis* CT443 polypeptide delivered as a mRNA was shown to elicit a potent antibody (e.g., IgG) response against *C. trachomatis* elementary bodies (EBs). The elicited antibodies were cross-reactive against EBs of different serovars of *C. trachomatis*. The inventors have demonstrated that the *C. trachomatis* CT443 polypeptides of the invention are suitable vaccine candidates.

In one aspect, the invention provides a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide. In another aspect, the invention provides a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT443 polypeptide. A *Chlamydia sp.* CT443 polypeptide for use in the present invention may elicit an antibody (e.g., IgG) response (e.g., an antigen-specific antibody response) in a subject. The elicited antibodies may bind *C. trachomatis* elementary bodies. The *Chlamydia sp.* CT443 polypeptide of the invention may elicit an antibody (IgG) response against more than one serovar of *C. trachomatis*, e.g., a cross-serovar response. Thus, the *Chlamydia sp.* CT443 polypeptide of the present invention may elicit a B cell (antibody) response against two or more serovars of *Chlamydia sp.* The response may be a protective antibody response.

A *Chlamydia sp.* CT443 polypeptide for use in the present invention may elicit a T cell response (e.g., antigen-specific T cell response) in a subject. In some embodiments, a *Chlamydia sp.* CT443 polypeptide for use in the present invention elicits IFN γ -producing T cells such as IFN γ -producing CD4+ T cells and/or IFN γ -producing CD8+ T cells in a subject.

CT443 (also referred to as outer membrane protein B (omcB or OMPB)) is an abundant outer membrane protein. It has been suggested that CT443 is an adhesin which promotes the interaction between the *C. trachomatis* elementary body and the host cell.²⁴ CT443, and in particular, its C-terminus, was shown to be immunogenic during human infection.^{25,26} It was also reported to be a protective antigen in mouse models.^{27,28}

A “*Chlamydia sp.* CT443 polypeptide” includes a mature form of a full-length native CT443 polypeptide of a *Chlamydia sp.* without its native signal peptide sequence and immunogenic variants thereof. An immunogenic variant of a native *Chlamydia sp.* CT443 polypeptide is capable of eliciting an immune response (e.g., an antigen specific immune response) in a subject, for example a T cell response and/or an antibody response. Immunogenic variants of a native *Chlamydia sp.* CT443 polypeptide include immunogenic fragments of a native *Chlamydia sp.* CT443 polypeptide. Immunogenic CT443 fragments include fragments of a native *Chlamydia sp.* CT443 polypeptide that are at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 325, at least 350, at least 375, at least 400, at least 425, at least 450, at least 475 or at least 500 amino acids long. Typically, the CT443 polypeptide of the invention is a full-length *Chlamydia sp.* CT443 polypeptide. In some embodiments, immunogenic variants exclude sequence motifs that are found in a subject (e.g., human) proteome, e.g., sequence motifs of 8 or more, 9 or more, 10 or more, 11 or more, 12

or more, 13 or more, 14 or more or 15 or more (e.g., 8 or more) amino acids in length. Excluded subject proteome sequence motifs are typically 8 or more amino acids in length. As explained above, this helps minimise unwanted cross-reactivity.

5 Exemplary *Chlamydia* sp. CT443 polypeptide sequences include a *Chlamydia trachomatis* CT443 polypeptide sequence (see SEQ ID NO: 507 which lacks native signal peptide sequence of the *Chlamydia trachomatis* CT443 polypeptide).

In some embodiments, the *Chlamydia* sp. CT443 polypeptide comprises the sequence of any one of SEQ ID NO: 507-508 (e.g., SEQ ID NO: 507) or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%,
10 at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the *Chlamydia* sp. CT443 polypeptide comprises a sequence having at least 90% identity to any one of SEQ ID NO 507-508 (e.g., SEQ ID NO: 507). In some embodiments, the *Chlamydia* sp. CT443 polypeptide comprises a sequence having at least 95% identity to any one of SEQ ID NO 507-508 (e.g., SEQ ID NO: 507).

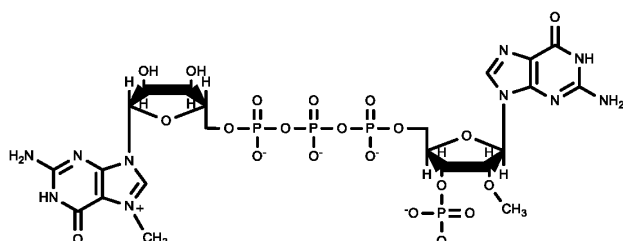
15 In some embodiments, a nucleic acid comprising a nucleotide sequence encoding a *Chlamydia* sp. CT443 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 707-710 (e.g., SEQ ID NO: 707) or a sequence that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the
20 nucleic acid comprises a nucleotide sequence that is least 85% identical to any one of SEQ ID NO: 707-710 (e.g., SEQ ID NO: 707). In some embodiments, the nucleic acid comprises a nucleotide sequence encoding a secretion signal peptide sequence as described herein, e.g., a secretion signal peptide sequence according to SEQ ID NO: 187. For example, the nucleic acid may comprise a sequence according to SEQ ID NO: 369.

25 In one embodiment, the nucleic acid is a mRNA comprising or consisting of (e.g., consisting of) the following structural elements:

- (1) a 5' cap;
- (2) a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- (3) a protein coding region having the nucleic acid sequence according to SEQ ID NO: 369 optionally
30 followed by a stop codon (e.g., TGA);
- (4) a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- (5) a polyA tail.

In one embodiment, the nucleic acid is a mRNA comprising or consisting of (e.g., consisting of) the following structural elements:

(1) a 5' cap with the following structure:



- 5 (2) a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- (3) a protein coding region having the nucleic acid sequence according to SEQ ID NO: 369;
- (4) a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- (5) a polyA tail.

10 In some embodiments, the 3' end of (1) bonds directly to the 5' end of (2) via a 3' to 5' phosphodiester linkage; the 3' end of (2) bonds directly to the 5' end of (3) via a 3' to 5' phosphodiester linkage; the 3' end of (3) bonds directly to the 5' end of (4) via a 3' to 5' phosphodiester linkage; and the 3' end of (4) bonds directly to the 5' end of (5) via a 3' to 5' phosphodiester linkage. In some embodiments, the mRNA is chemically modified and the chemical modification comprises N1-methylpseudouridine in place of every uridine. The mRNA may be encapsulated in a LNP.

15 **CT584**

The inventors have demonstrated that mRNAs encoding *C. trachomatis* CT584 polypeptide and a recombinant *C. trachomatis* CT584 protein elicited IFN γ -producing T cells. Furthermore, *C. trachomatis* CT584 polypeptide was shown to elicit a robust IgG response when delivered either as a mRNA expressing the antigen or in the form of a recombinant protein. *C. trachomatis* CT584 polypeptide delivered as a

20 mRNA was shown to elicit a potent antibody (e.g., IgG) response against *C. trachomatis* elementary bodies (EBs). The elicited antibodies were cross-reactive against EBs of different serovars of *C. trachomatis*. The inventors have demonstrated that the *C. trachomatis* CT584 polypeptides of the invention are suitable vaccine candidates.

In one aspect, the invention provides a nucleic acid that comprises a nucleotide sequence encoding a

25 *Chlamydia sp.* CT584 polypeptide. In another aspect, the invention provides a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT584 polypeptide. A *Chlamydia sp.* CT584 polypeptide for use in the present invention may elicit an antibody (e.g., IgG) response (e.g., an antigen-specific antibody

response) in a subject. The elicited antibodies may bind *C. trachomatis* elementary bodies. The *Chlamydia sp.* CT584 polypeptide of the invention may elicit an antibody (IgG) response against more than one serovar of *C. trachomatis*, e.g., a cross-serovar response. Thus, the *Chlamydia sp.* CT584 polypeptide of the present invention may elicit a B cell (antibody) response against two or more serovars of *Chlamydia sp.* The response may be a protective antibody response.

A *Chlamydia sp.* CT584 polypeptide for use in the present invention may elicit a T cell response (e.g., antigen-specific T cell response) in a subject. In some embodiments, a *Chlamydia sp.* CT584 polypeptide for use in the invention elicits IFN γ -producing T cells such as IFN γ -producing CD4⁺ T cells and/or IFN γ -producing CD8⁺ T cells in a subject.

CT584 is a putative tip protein in a Type III secretion system (based on computational structure prediction and homology search study on *C. trachomatis*).²⁹ It has high sequence homology with proteins from other *Chlamydiae*, but it does not share homology with proteins from other bacterial genera.³⁰ It is also universally conserved amongst all *C. trachomatis* serovars. CT584 is therefore a promising target antigen for generating cross-serovar immune responses.

A “*Chlamydia sp.* CT584 polypeptide” includes a full-length native CT584 polypeptide of a *Chlamydia sp.* and immunogenic variants thereof. An immunogenic variant of a *Chlamydia sp.* CT584 polypeptide is capable of eliciting an immune response in a subject, for example a T cell response and/or an antibody response (e.g., an antigen-specific T cell and/or antibody responses). Immunogenic variants of CT584 include immunogenic fragments of a native *Chlamydia sp.* CT584 polypeptide. Immunogenic CT584 fragments include fragments of a native *Chlamydia sp.* CT584 polypeptide that are at least 50, at least 75, at least 100, at least 125, at least 150, at least 175 amino acids long. Typically, the CT584 polypeptide of the invention is a full-length *Chlamydia sp.* CT584 polypeptide. In embodiments, immunogenic variants exclude sequence motifs that are found in a subject (e.g., human) proteome, e.g., sequence motifs of 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more or 15 or more amino acids in length. Excluded subject proteome (e.g., human proteome) sequence motifs are typically 8 or more amino acids in length. As explained above, this helps minimise unwanted cross-reactivity.

Exemplary *Chlamydia sp.* CT584 polypeptide sequences include a *Chlamydia trachomatis* CT584 polypeptide sequence (see SEQ ID NO: 509). Typically, the *Chlamydia sp.* CT584 polypeptide of the invention comprises a single amino acid substitution at a position corresponding to residue 11 of SEQ ID NO: 509 (e.g., a N to Q substitution).

In some embodiments, the *Chlamydia sp.* CT584 polypeptide comprises the sequence of any one of SEQ ID NO: 509-512 (SEQ ID NO: 510) or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the *Chlamydia sp.* CT584

polypeptide comprises a sequence having at least 90% identity to any one of SEQ ID NO 509-512 (e.g., SEQ ID NO: 510). In some embodiments, the *Chlamydia sp.* CT584 polypeptide comprises a sequence having at least 95% identity to any one of SEQ ID NO 509-512 (e.g., SEQ ID NO: 510).

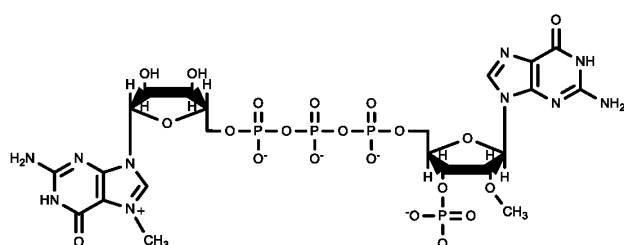
In some embodiments, a nucleic acid comprising a nucleotide sequence encoding a *Chlamydia* sp. CT584 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 711-718 (e.g., SEQ ID NO: 715) or a sequence that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the nucleic acid comprises a nucleotide sequence that is least 85% identical to any one of SEQ ID NO: 711-718 (e.g., SEQ ID NO: 715). In some embodiments, the nucleic acid comprises a nucleotide sequence encoding a secretion signal peptide sequence as described herein, e.g., a secretion signal peptide sequence according to SEQ ID NO: 187. For example, the nucleic acid may comprise a sequence according to SEQ ID NO: 377.

In one embodiment, the nucleic acid is a mRNA comprising or consisting of (e.g., consisting of) the following structural elements:

- (1) a 5' cap;
- (2) a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- (3) a protein coding region having the nucleic acid sequence according to SEQ ID NO: 377 optionally followed by a stop codon (e.g., TGA);
- (4) a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- (5) a polyA tail.

In one embodiment, the nucleic acid is a mRNA comprising or consisting of (e.g., consisting of) the following structural elements:

- (1) a 5' cap with the following structure:



- (2) a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;

- (3) a protein coding region having the nucleic acid sequence according to SEQ ID NO: 377;
- (4) a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- (5) a polyA tail.

In some embodiments, the 3' end of (1) bonds directly to the 5' end of (2) via a 3' to 5' phosphodiester linkage; the 3' end of (2) bonds directly to the 5' end of (3) via a 3' to 5' phosphodiester linkage; the 3' end of (3) bonds directly to the 5' end of (4) via a 3' to 5' phosphodiester linkage; and the 3' end of (4) bonds directly to the 5' end of (5) via a 3' to 5' phosphodiester linkage. In some embodiments, the mRNA is chemically modified and the chemical modification comprises N1-methylpseudouridine in place of every uridine. The mRNA may be encapsulated in a LNP.

CT600

The inventors have demonstrated that *C. trachomatis* polypeptide CT600 elicited a robust IgG response when delivered either as a mRNA expressing the antigen or in the form of a recombinant protein. The inventors have also shown that a mRNA encoding *C. trachomatis* CT600 polypeptide and a recombinant *C. trachomatis* CT600 protein were able to elicit IFN γ -producing T cells.

In one aspect, the invention provides a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide. In another aspect, the invention provides a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT600 polypeptide. A *Chlamydia sp.* CT600 polypeptide for use in the present invention may elicit an antibody (e.g., IgG) response (e.g., an antigen-specific antibody response) in a subject. *C. trachomatis* CT600 polypeptide delivered as a mRNA was shown to elicit a potent antibody (e.g., IgG) response against *C. trachomatis* elementary bodies (EBs). The elicited antibodies were cross-reactive against EBs of different serovars of *C. trachomatis*. The inventors have demonstrated that the *C. trachomatis* C600 polypeptides of the invention are suitable vaccine candidates.

A *Chlamydia sp.* CT600 polypeptide for use in the present invention may elicit a T cell response (e.g., antigen-specific T cell response) in a subject. In some embodiments, a *Chlamydia sp.* CT600 polypeptide for use in the invention elicits IFN γ -producing T cells such as IFN γ -producing CD4+ T cells and/or IFN γ -producing CD8+ T cells in a subject.

CT600 is a *Chlamydia sp.* Peptidoglycan-Associated Lipoprotein (Pal), which is thought to form a cross-bridge between the peptidoglycan and outer membrane.

A "*Chlamydia sp.* CT600 polypeptide" includes a mature form of a full-length native CT600 polypeptide of a *Chlamydia sp.* without its native signal peptide sequence. An immunogenic variant of a native *Chlamydia sp.* CT600 polypeptide is capable of eliciting an immune response (e.g., an antigen specific immune response) in a subject, for example a T cell response and/or an antibody response. Immunogenic

variants of a native *Chlamydia sp.* CT600 polypeptide include immunogenic fragments of a native *Chlamydia sp.* CT600 polypeptide. Immunogenic CT600 fragments include fragments of a native *Chlamydia sp.* CT600 polypeptide that are at least 50, at least 75, at least 100, at least 125 or at least 150 amino acids long. Preferred immunogenic fragments of a *Chlamydia sp.* CT600 polypeptide include fragments lacking the N-terminal cysteine-rich region of a *Chlamydia sp.* CT600 polypeptide (e.g., fragments lacking 13 amino acids that immediately follow the native signal peptide of a *Chlamydia sp.* CT600 polypeptide). In embodiments, immunogenic fragments exclude sequence motifs that are found in a subject (e.g., human) proteome, e.g., sequence motifs of 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more or 15 or more amino acids in length. Excluded subject proteome (e.g., human proteome) sequence motifs are typically 8 or more amino acids in length. As explained above, this helps minimise unwanted cross-reactivity.

Exemplary *Chlamydia sp.* CT600 polypeptide sequences include a *Chlamydia trachomatis* CT600 polypeptide sequence in SEQ ID NO: 513 which lacks a native signal peptide sequence of the *Chlamydia trachomatis* CT600 polypeptide and the 13 amino acid-long region which immediately follows the signal peptide.

In some embodiments, the *Chlamydia sp.* CT600 polypeptide comprises the sequence of SEQ ID NO: 513 or 514 or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the *Chlamydia sp.* CT600 polypeptide comprises a sequence having at least 90% identity to any one of SEQ ID NO 513-514. In some embodiments, the *Chlamydia sp.* CT600 polypeptide comprises a sequence having at least 95% identity to any one of SEQ ID NO 513-514.

In some embodiments, a nucleic acid comprising a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 719-722 or a sequence that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the nucleic acid comprises a nucleotide sequence that is least 85% identical to any one of SEQ ID NO: 719-722.

CT812

The inventors have demonstrated that mRNAs encoding *C. trachomatis* CT812 polypeptide and a recombinant *C. trachomatis* CT812 protein induced IFN γ -producing T cells.

In one aspect, the invention provides a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide. In another aspect, the invention provides a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT812 polypeptide. A *Chlamydia sp.* CT812 polypeptide for use

in the present invention may elicit an antibody (e.g., IgG) response (e.g., an antigen-specific antibody response) in a subject. A *Chlamydia sp.* CT812 polypeptide for use in the present invention may elicit a T cell response (e.g., antigen-specific T cell response) in a subject. In some embodiments, a *Chlamydia sp.* CT812 polypeptide for use in the invention elicits IFN γ -producing T cells such as IFN γ -producing CD4+ T cells and/or IFN γ -producing CD8+ T cells in a subject.

CT812 (also referred to as Polymorphic Membrane Protein D (or PmpD)) is an autotransporter protein anchored in the outer membrane of *Chlamydia sp.* bacteria. CT812 is a proposed adhesin protein. Mature CT812 comprises a passenger domain and a beta barrel domain.³² Immunisation with recombinant CT812 protein has been shown to be protective against *C. trachomatis* serovar D in mice.³¹

A “*Chlamydia sp.* CT812 polypeptide” as used herein includes a mature form of a full-length native CT812 polypeptide of a *Chlamydia sp.* without its native signal peptide sequence and immunogenic variants thereof. An immunogenic variant of a *Chlamydia sp.* CT812 polypeptide is capable of eliciting an immune response (e.g., an antigen specific immune response) in a subject, for example a T cell response and/or an antibody response. Immunogenic variants of a native *Chlamydia sp.* CT812 polypeptide include immunogenic fragments of a native *Chlamydia sp.* CT812 polypeptide. Immunogenic CT812 fragments include fragments of a native *Chlamydia sp.* CT812 polypeptide that are at least 50, at least 75, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1050, at least 1100 or at least 1150 amino acids long. In some embodiments, immunogenic fragments of a *Chlamydia sp.* CT812 polypeptide include fragments of the amino acid sequence in SEQ ID NO: 515. In some embodiments, immunogenic fragments may be fragments of the passenger domain, e.g., fragments comprising residues 52-1003 of SEQ ID NO: 515 or residues 52-1179 of SEQ ID NO: 515. In some embodiments, immunogenic fragments comprise the amino acid sequence according to SEQ ID NOs: 516 or 518. In some embodiments, fragments of the passenger domain may be at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1050, at least 1100 or at least 1150 amino acids long. In embodiments, immunogenic fragments exclude sequence motifs that are found in a subject (e.g., human) proteome, e.g., sequence motifs of 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more or 15 or more amino acids in length. Excluded subject proteome (e.g., human proteome) sequence motifs are typically 8 or more amino acids in length. As explained above, this helps minimise unwanted cross-reactivity.

Exemplary *Chlamydia sp.* CT812 polypeptide sequences include a *Chlamydia trachomatis* CT812 polypeptide sequence in SEQ ID NO: 515 which lacks a native signal peptide sequence of the *Chlamydia trachomatis* CT812 polypeptide. *Chlamydia sp.* CT812 polypeptide sequences also include SEQ ID NOs 516 and 518.

In some embodiments, the *Chlamydia sp.* CT812 polypeptide comprises the sequence of any one of SEQ ID NO: 515-535 or a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the *Chlamydia sp.* CT812 polypeptide comprises a sequence having at least 90% identity to any one of SEQ ID NO 515-535. In some embodiments, the *Chlamydia sp.* CT812 polypeptide comprises a sequence having at least 95% identity to any one of SEQ ID NO 515-535.

In some embodiments, a nucleic acid comprising a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 723-762 or a sequence that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the nucleic acid comprises a nucleotide sequence that is least 85% identical to any one of SEQ ID NO: 723-762.

Variants of modified MOMP polypeptides, chimeric MOMP VD polypeptides and non-MOMP antigen polypeptides

The sequences of the modified MOMP polypeptide, the chimeric *Chlamydia sp.* MOMP VD polypeptides, *Chlamydia sp.* CT443 polypeptides, *Chlamydia sp.* CT584 polypeptides, *Chlamydia sp.* CT600 polypeptides and/or *Chlamydia sp.* CT812 polypeptides as described herein may comprise one or more mutations or modifications.

In some embodiments, the modified MOMP polypeptides, the chimeric *Chlamydia sp.* MOMP VD polypeptides, *Chlamydia sp.* CT443 polypeptides, *Chlamydia sp.* CT584 polypeptides, *Chlamydia sp.* CT600 polypeptides and/or *Chlamydia sp.* CT812 polypeptides as described herein may comprise one or more conservative amino acid substitutions.

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In some embodiments, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

Mutation of cysteine residues

One or more cysteine residues in the polypeptides described herein may be mutated by single amino acid substitutions, e.g., to a serine residue. Cysteine residues are involved in disulphide bridge formation and thus cysteine mutations may limit polypeptide multimerisation.

- 5 In some embodiments, a modified MOMP polypeptide described herein comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide. In some embodiments, a chimeric MOMP VD polypeptide described herein may comprise a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.*
- 10 MOMP polypeptide. Conserved domains of a native *C. trachomatis* serovar E MOMP polypeptides comprise Cys residues at positions C48, C51, C55, C124, C137, C204, C206, C229 and/or C357 of SEQ ID NO: 2. Conserved domains of native MOMP polypeptides of *Chlamydia sp.* may comprise cysteine residues at positions corresponding to the residues set out for the native MOMP polypeptide of serovar E of *C. trachomatis*.
- 15 In some embodiments, the *Chlamydia sp.* CT443 polypeptide described herein comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a native *Chlamydia sp.* CT443 polypeptide. In some embodiments, the *Chlamydia sp.* CT443 polypeptide comprises one or more single amino acid substitutions at positions corresponding to C57, C100, C111, C156, C157, C164, C166, C175, C181, C191, C194, C252, C268, C285, C299, C341, C380, C383, C386, C389, C405,
- 20 C410, C414 and/or C424 of SEQ ID NO: 507. In some embodiments, the *Chlamydia sp.* CT443 polypeptide comprises one or more single amino acid substitutions at positions corresponding to C57, C100, C111, C156, C157, C164, C166, C175, C181, C191, C194, C252, C268, C285, C299, C341, C380, C386, C405, C410, C414 and/or C424 of SEQ ID NO: 507. Typically, however, the *Chlamydia sp.* CT443 polypeptide described herein does not comprise a single amino acid substitution at any positions corresponding to a
- 25 cysteine residue in a native *Chlamydia sp.* CT443 polypeptide.

- In some embodiments, the *Chlamydia sp.* CT584 polypeptide described herein comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a native *Chlamydia sp.* CT584 polypeptide. In some embodiments, the *Chlamydia sp.* CT584 polypeptide comprises one or more single amino acid substitutions at positions corresponding to C44, C114 and/or C138 of SEQ
- 30 ID NO: 509.

In some embodiments, the *Chlamydia sp.* CT600 polypeptide described herein comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a native *Chlamydia sp.* CT600 polypeptide. In some embodiments, the *Chlamydia sp.* CT600 polypeptide comprises

one or more (e.g., all) single amino acid substitutions at positions corresponding to C1, C5, C10, and/or C13 of SEQ ID NO: 844.

In some embodiments, the *Chlamydia sp.* CT812 polypeptide described herein comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a native *Chlamydia sp.* CT812 polypeptide. In some embodiments, the *Chlamydia sp.* CT812 polypeptide comprises one or more (e.g., all) single amino acid substitutions at positions corresponding to C1, C96, C160, C169, C179, C187, C233, C291, C300, C331, C348, C365, C401, C432, C560, C570, C571, C641, C665, C756, C838, C859, C963, C980 and/or C1477 of SEQ ID NO: 515. In some embodiments, the *Chlamydia sp.* CT812 polypeptide comprises one or more (e.g., all) single amino acid substitutions at positions corresponding to C96, C169, C291, C300, C331, C348, C365, C401, C432, C560, C570, C571, C756, C838, C859, C963 and/or C980 of SEQ ID NO: 515.

Mutation of glycosylation sites

Glycosylation may occur in eukaryotic cells but not in prokaryotic cells. "Glycosylation" as used herein refers to the addition of a saccharide unit to a protein. In particular, N-linked glycosylation is the attachment of glycan to an amide nitrogen of an asparagine (Asn; N) residue of a protein. Glycosylation can occur at any asparagine residue in a protein that is accessible to and recognised by glycosylating enzymes following translation of the protein, and is most common at accessible asparagines that are part of an NXS/T motif, wherein the second amino acid residue following the asparagine is a serine (Ser; S) or threonine (Thr; T). O-linked glycosylation is the attachment of a glycan to the oxygen atom of serine (Ser) or threonine (Thr) residue in a protein. The process of attachment results in a glycosylated protein. This glycan may be a polysaccharide. A non-human glycosylation pattern can render a polypeptide undesirably reactogenic when used to elicit antibodies. Additionally, glycosylation of a polypeptide that is not normally glycosylated (such as polypeptides described herein) may alter its immunogenicity. For example, glycosylation can mask important immunogenic epitopes within a protein. Thus, to reduce or eliminate glycosylation, either asparagine residues or serine/threonine residues can be modified, for example, by substitution to another amino acid.

In certain embodiments, one or more of the modified MOMP polypeptide, chimeric *Chlamydia sp.* MOMP VD polypeptide, *Chlamydia sp.* CT443 polypeptide, *Chlamydia sp.* CT584 polypeptide, *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide as described herein comprises at least one mutated glycosylation site, preferably at least one mutated N-linked glycosylation site and/or at least one O-linked glycosylation site. In some embodiments, one or more N-glycosylation sites in one or more of the modified MOMP polypeptide, chimeric *Chlamydia sp.* MOMP VD polypeptide, *Chlamydia sp.* CT443 polypeptide, *Chlamydia sp.* CT584 polypeptide, *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide as described herein are removed. The removal of an N-glycosylation site may decrease glycosylation of the polypeptide. The removal of a glycosylation site may decrease glycosylation of the

polypeptide. In some embodiments, one or more of the modified MOMP polypeptide, the chimeric *Chlamydia sp.* MOMP VD polypeptides, *Chlamydia sp.* CT443 polypeptide, *Chlamydia sp.* CT584 polypeptide, *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide as described herein has decreased glycosylation relative to the respective native polypeptide. The removal of N-glycosylation sites may eliminate N-glycosylation of the polypeptide. The removal of O-glycosylation sites may eliminate O-glycosylation of the polypeptide.

In certain embodiments, the modification comprises a substitution of one or more of an N, S, and T amino acid in an NXS/T sequence motif, wherein X corresponds to any amino acid. In some embodiments, the modification comprises a substitution of one or more serine (Ser) or threonine (Thr) residue(s) in a protein. In some embodiments, an N, S, or T amino acid is substituted with a conservative amino acid substitution. Typically, an N amino acid may be substituted with a Q, S, K or A amino acid.

In some embodiments, the chimeric MOMP VD polypeptide described herein comprises a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to an N-glycosylation site in a native *Chlamydia sp.* MOMP polypeptide. N-glycosylation sites in a native *Chlamydia sp.* MOMP polypeptide may be in one or more MOMP VD domains. N-glycosylation sites may be present in MOMP VD1 of serovar E (position 9 of SEQ ID NO: 9), MOMP VD1 of serovar F (position 11 of SEQ ID NO: 13), MOMP VD1 of serovar G (position 11 of SEQ ID NO: 17), MOMP VD2 of serovar E (position 6 and/or 17 of SEQ ID NO: 10), MOMP VD2 of serovar F (position 4 and/or 21 of SEQ ID NO: 14), MOMP VD2 of serovar D (position 17 of SEQ ID NO: 6); MOMP VD2 of serovar G (position 4 and/or 21 of SEQ ID NO: 18), MOMP VD4 of serovar E (position 14 in SEQ ID NO: 12); MOMP VD4 of serovar G (position 14 in SEQ ID NO: 20); MOMP VD4 of serovar D (position 14 in SEQ ID NO: 8) and/or MOMP VD4 of serovar F (position 14 in SEQ ID NO: 16), wherein serovars D-G are of *C. trachomatis*. In some embodiments, the chimeric MOMP VD polypeptide comprises (i) a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar G; and (ii) a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G; and (iii) a MOMP VD3 sequence from serovar F; and (iv) a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F, wherein the serovars D, E, F or G are of *C. trachomatis*. Typically, the chimeric MOMP VD polypeptide comprises a single amino acid substitution at each of the amino acid residues corresponding to position 9 of SEQ ID NO: 9 (*e.g.*, N to A substitution), position 11 of SEQ ID NO: 17 (*e.g.*, T to A substitution), position 17 of SEQ ID NO: 6 (*e.g.*, S to A substitution), positions 4 and 21 of SEQ ID NO: 18 (*e.g.*, N to A and S to A substitutions, respectively), position 14 of SEQ ID NO: 8 (*e.g.*, a T to A substitution) and position 14 in SEQ ID NO: 16 (*e.g.*, T to A substitution).

In some embodiments, the *Chlamydia sp.* CT443 polypeptide described herein comprises a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to an N-glycosylation site in a native *Chlamydia sp.* CT443 polypeptide. In some embodiments, the *Chlamydia sp.* CT443 polypeptide comprises

one or more (e.g., all) single amino acid substitutions at positions corresponding to residues 18, 29 and 435 of SEQ ID NO: 507.

5 In some embodiments, the *Chlamydia sp.* CT584 polypeptide described herein comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to an N-glycosylation site in a native *Chlamydia sp.* CT584 polypeptide. Typically, the *Chlamydia sp.* CT584 polypeptide comprises a single amino acid substitution at a position corresponding to residue 11 of SEQ ID NO: 509 (e.g., a N to Q substitution).

10 In some embodiments, the *Chlamydia sp.* CT600 polypeptide described herein comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to an N-glycosylation site in a native *Chlamydia sp.* CT600 polypeptide. In some embodiments, the *Chlamydia sp.* CT600 polypeptide comprises one or two single amino acid substitutions at positions corresponding to residues 48 and 65 of SEQ ID NO: 513.

15 In some embodiments, the *Chlamydia sp.* CT812 polypeptide described herein comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to an N-glycosylation site in a native *Chlamydia sp.* CT812 polypeptide.

Secretion signal peptide sequences

The modified MOMP polypeptide, the chimeric *Chlamydia sp.* MOMP VD polypeptide, *Chlamydia sp.* CT443 polypeptide, *Chlamydia sp.* CT584 polypeptide, *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide as described herein may comprise a secretion signal peptide sequence.

20 The secretion signal peptide may be cleaved in post-translation processing of the *Chlamydia sp.* polypeptides described herein. The mature form of the *Chlamydia sp.* polypeptide may therefore not comprise the secretion signal peptide sequence. However, a nucleotide sequence encoding a secretion signal peptide sequence may be present in nucleic acids described herein encoding the *Chlamydia sp.* polypeptides described herein.

25 The modified MOMP polypeptide, the chimeric *Chlamydia sp.* MOMP VD polypeptide, *Chlamydia sp.* CT443 polypeptide, *Chlamydia sp.* CT584 polypeptide, *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide as described herein may comprise a secretion signal peptide sequence of the respective native *Chlamydia sp.* polypeptide. This may be advantageous if the *Chlamydia sp.* polypeptides are expressed in a prokaryotic cell as recombinant proteins.

30 In some embodiments, the modified MOMP polypeptide, the chimeric *Chlamydia sp.* MOMP VD polypeptide, *Chlamydia sp.* CT443 polypeptide, *Chlamydia sp.* CT584 polypeptide, *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide as described herein may comprise viral or eukaryotic (e.g., human) secretion signal peptide (SS) sequences. The use of viral or eukaryotic secretion signal peptide

sequences attached to a polypeptide described herein may offer numerous advantages for immunogenic compositions. When expressed from an mRNA, especially in a eukaryotic cell, a polypeptide of the invention comprising a SS sequence may have increased extracellular expression relative to the polypeptide without the SS sequence. The increased extracellular expression may promote higher immunogenicity and by extension, better vaccine efficacy.

Viral SS sequences may be found in publicly accessible databases (e.g., the NCBI or UniProt databases) which include an annotated viral polypeptide sequence and identify the start and end position of an experimentally validated SS.

In certain embodiments, the SS sequence as well as the location of the SS sequence cleavage site for a given known input polypeptide sequence may be predicted by using the SignalP algorithm. The SignalP algorithm (and more particularly SignalP v6.0) is described in further detail in Armenteros et al. (Nature Biotechnology. 37: 420-423. 2019), Teufel et al. (Nature Biotechnology. 40: 1023-1025. 2022), and <https://services.healthtech.dtu.dk/services/SignalP-6.0/>, each of which is incorporated herein by reference in their entirety. The strength of the prediction is assessed based on a cumulative rank score that considers the likelihood of detecting canonical features of the signal sequence (SS likelihood score) and the probability of cleavage at the cleavage site (cleavage probability score). In certain embodiments, the viral secretion signal peptide has a SignalP cleavage probability score of at least 0.8, at least 0.85, at least 0.90 or at least 0.95, as determined using SignalP 6.0. In some embodiments, the viral secretion signal peptide has a SignalP signal peptide likelihood score of at least 0.8, at least 0.85, at least 0.90 or at least 0.95, as determined using SignalP 6.0.

In certain embodiments, the SS sequence is a viral SS sequence. In certain embodiments, the viral secretion signal peptide sequence is derived from a viral sequence in a virus able to infect humans. The phrase “influenza”, “SARS CoV-2”, “varicella-zoster virus (VZV)”, “measles”, “rubella”, “rabies,” “Ebola,” and “smallpox” preceding the phrase “secretion signal peptide sequence” indicates that the secretion signal peptide was derived from the virus corresponding to that name.

In certain embodiments, the viral secretion signal peptide is derived from a viral sequence selected from the group consisting of: an influenza secretion signal peptide sequence, a SARS CoV-2 secretion signal peptide sequence, a varicella-zoster virus (VZV) secretion signal peptide sequence, a measles secretion signal peptide sequence, a rubella secretion signal peptide sequence, a mumps secretion signal peptide sequence, an Ebola secretion signal peptide sequence, a rabies secretion signal peptide sequence, and a smallpox secretion signal peptide sequence. These particular signal peptides are derived from viral sequences in viruses which have been administered to humans as vaccines (live-attenuated, inactivated or mRNA), with demonstrated strong safety profiles.

In certain embodiments, the viral secretion signal peptide is selected from the group consisting of: an influenza hemagglutinin (HA) secretion signal peptide sequence, a SARS CoV-2 spike secretion signal peptide sequence, a VZV gB secretion signal peptide sequence, a VZV gE secretion signal peptide sequence, a VZV gI secretion signal peptide sequence, a VZV gK secretion signal peptide sequence, a measles F-protein secretion signal peptide sequence, a rubella E1 protein secretion signal peptide sequence, a rubella E2 protein secretion signal peptide sequence, a mumps F-protein secretion signal peptide sequence, an Ebola GP protein secretion signal peptide sequence, a rabies virus glycoprotein (Rabies G) secretion signal peptide sequence, and a smallpox 6kDa IC protein secretion signal peptide sequence.

In certain embodiments, the viral secretion signal peptide comprises an HA secretion signal peptide sequence from influenza A or influenza B, preferably from influenza A.

Exemplary viral secretion signal peptide amino acid sequences of the disclosure are shown below in Table 2. Exemplary viral secretion signal peptide amino acid sequences derived from Influenza A or B of the disclosure are shown below in Table 2.1.

Table 2 – Viral Secretion Signal Peptide (SS) Amino Acid Sequences

NAME OF THE PROTEIN	ORGANISM	STRAIN	SEQUENCE OF THE SS
HA (H1N1)	Influenza virus	A/New Caledonia/20/1999	MKAKLLVLLCTFTATYA (SEQ ID NO: 187)
HA (H1N1pdm)	Influenza virus	A/California/7/2009	MKAILVLLYTFATANA (SEQ ID NO: 188)
HA (H3N2)	Influenza virus	A/Moscow/10/1999	MKTIALSYILCLVFA (SEQ ID NO: 193)
HA B	Influenza virus	B/Phuket/3073/2013	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
Spike	SARS CoV-2	Wuhan-1	MFVFLVLLPLVS (SEQ ID NO: 195)
Spike	SARS CoV-2	Wuhan-1 (long version)	MFLTTKRTMFVFLVLLPLV S (SEQ ID NO: 196)
gB	VZV	Oka strain	MSPCGYYSKWRNRDRPEYR RNLRFRRFFSSIHPNAAAGSG FNGPGVFITSVTGVWLCFLCI FSMFVTAVVS (SEQ ID NO: 803)
gE	VZV	Oka strain	MGTVNKPVVGVLGMFGIIT GTLRITNPVRA (SEQ ID NO: 804)
gI	VZV	Oka strain	MFLIQCLISAVIFYIQVTNA (SEQ ID NO: 805)
gK	VZV	Oka strain	MQALGIKTEHFIIIMCLLSGH A (SEQ ID NO: 806)
F	Measles	Edmonston-Zagreb strain	MGLKVNVSIAFMAVLLTLQ TPTG

			(SEQ ID NO: 807)
E1	Rubella	RA27/3 strain	MGAAAALTAVVLQGYNPPA YG (SEQ ID NO: 808)
E2	Rubella	RA27/3 strain	MGAPQAFLAGLLAAVAVG TARA (SEQ ID NO: 809)
F	Mumps	Miyahara strain	MKVFLVTCLGFAVFSSVC (SEQ ID NO: 810)
GP	Ebola	Mayinga-76 strain	MGVTGILQLPRDRFKRTSFF LWVILFQRTFS (SEQ ID NO: 811)
6kDa IC	Smallpox	Germany 91-3 strain	MRSLLIFLLFPSIIYS (SEQ ID NO: 812)
Rabies G	Rabies	Rabies Pasteur strain	MVPQALLFVPLLVFPLCFG (SEQ ID NO: 845)

Table 2.1 – Influenza virus A and B Specific Viral Secretion Signal Peptide (SS) Amino Acid Sequences

STRAIN NAME OR ID	SEQUENCE OF THE SS
A/Beijing/262/95 (H1N1)-like virus	MKAKLLVLLCTFTATYA (SEQ ID NO: 187)
A/Brisbane/02/2018 (H1N1)pdm09-like virus	MKAILVVLLYTFTTANA (SEQ ID NO: 846)
A/Brisbane/59/2007 (H1N1)-like virus	MKVKLLVLLCTFTATYA (SEQ ID NO: 847)
A/California/7/2009 (H1N1)-like virus	MKAILVVLLYTTFATANA (SEQ ID NO: 188)
A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like virus	MKAILVVLLYTFTTANA (SEQ ID NO: 846)
A/Hawaii/70/2019 (H1N1)pdm09-like virus	MKAILVVLLYTFTTANA (SEQ ID NO: 846)
A/Michigan/45/2015 (H1N1)pdm09-like virus	MKAILVVLLYTFTTANA (SEQ ID NO: 846)
A/New Caledonia/20/99 (H1N1)-like virus	MKAKLLVLLCTFTATYA (SEQ ID NO: 187)
A/Solomon Islands/3/2006 (H1N1)-like virus	MKVKLLVLLCTFTATYA (SEQ ID NO: 847)
A/Sydney/5/2021 (H1N1)pdm09-like virus	MKAILVVMLYTFTTANA (SEQ ID NO: 848)
A/Victoria/2570/2019 (H1N1)pdm09-like virus	MKAILVVMLYTFTTANA (SEQ ID NO: 848)

A/Victoria/4897/2022 (H1N1)pdm09-like virus	MKAILVVMLYTFTTANA (SEQ ID NO: 848)
A/Wisconsin/588/2019 (H1N1)pdm09-like virus	MKAILVVMLYTFTTANA (SEQ ID NO: 848)
A/Wisconsin/67/2022 (H1N1)pdm09-like virus	MKAILVVMLYTFTTANA (SEQ ID NO: 848)
H1N1 CONSENSUS SEQ #1 (without substitutions)	MKAILVVLLYTFTTANA (SEQ ID NO: 846)
A/Brisbane/10/2007 (H3N2)-like virus	MKTIIALSYILCLVFT (SEQ ID NO: 849)
A/California/7/2004 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Cambodia/e0826360/20 20 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Darwin/6/2021 (H3N2)- like virus	MKTIIALSNILCLVFA (SEQ ID NO: 850)
A/Darwin/9/2021 (H3N2)- like virus	MKTIIALSNILCLVFA (SEQ ID NO: 850)
A/Fujian/411/2002 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Hong Kong/2671/2019 (H3N2)-like virus	MKAIIALSNILCLVFA (SEQ ID NO: 851)
A/Hong Kong/45/2019 (H3N2)-like virus	MKAIIALSNILCLVFA (SEQ ID NO: 851)
A/Hong Kong/4801/2014 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Kansas/14/2017 (H3N2)-like virus	MKTIIALSCILCLVFA (SEQ ID NO: 852)
A/Moscow/10/99 (H3N2)- like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Perth/16/2009 (H3N2)- like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Singapore/INFIMH-16- 0019/2016 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/South Australia/34/2019 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Switzerland/8060/2017 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Switzerland/9715293/2 013 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Sydney/5/97 (H3N2)- like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)

A/Texas/50/2012 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Victoria/361/2011 (H3N2)-like virus	MKTIIALSHILCLVFA (SEQ ID NO: 853)
A/Wellington/1/2004 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
A/Wisconsin/67/2005 (H3N2)-like virus	MKTIIALSYILCLVFA (SEQ ID NO: 193)
H3N2 CONSENSUS SEQ #1 (without substitutions)	MKTIIALSYILCLVFA (SEQ ID NO: 193)
B/Austria/1359417/2021 (B/Victoria lineage)-like	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Brisbane/60/2008-like virus	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage)	MKAIIVLLMVVTSSA (SEQ ID NO: 854)
B/Hong Kong/330/2001-like virus	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Malaysia/2506/2004-like virus	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Washington/02/2019 (B/Victoria lineage)-like virus	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Beijing/184/93-like virus	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Florida/4/2006-like virus	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Massachusetts/2/2012-like virus	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Phuket/3073/2013-like virus	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
B/Sichuan/379/99-like virus	MEAIIVLLMVVTSNA (SEQ ID NO: 855)
INFLUENZA B VICTORIA/YAMAGATA A CONSENSUS SEQUENCE (without substitutions)	MKAIIVLLMVVTSNA (SEQ ID NO: 194)
H1N1 CONSENSUS SEQ #2 (with substitutions)	MKX₁X₂LX₃VX₄LX₅TFX₆X₇X₈X₉A X₁ is selected from A and V; X₂ is selected from I and K; X₃ is selected from V and L; X₄ is selected from L and M; X₅ is selected from Y and C; X₆ is selected from T and A; X₇ is selected from T and A; X₈ is selected from A and T; and X₉ is selected from N and Y (SEQ ID NO: 856)

H3N2 CONSENSUS SEQ #2 (with substitutions)		MKX ₁ IIALSX ₂ ILCLVFX ₃ X ₁ is selected from T and A; X ₂ is selected from Y, N, C, and H; and X ₃ is selected from T and A (SEQ ID NO: 857)
INFLUENZA VICTORIA CONSENSUS SEQ (with substitutions)	B	MKAIIVLLMVVTSX ₁ A X ₁ is selected from S and N (SEQ ID NO: 858)
INFLUENZA YAMAGATA CONSENSUS SEQ (with substitutions)	B	MX ₁ AIIVLLMVVTSNA X ₁ is selected from K and E (SEQ ID NO: 859)

The secretion signal peptide sequence may be positioned at the N terminus or the C terminus (e.g., at the N terminus) of a polypeptide described herein.

In certain embodiments, the SS amino acid sequence is encoded by a codon-optimized polynucleotide sequence.

In certain embodiments, the viral secretion signal peptide is derived from a viral sequence in a virus able to infect humans.

In certain embodiments, the viral secretion signal peptide is derived from a viral sequence selected from the group consisting of: an influenza secretion signal peptide sequence, and a non-influenza secretion signal peptide sequence selected from the group consisting of a SARS CoV-2 secretion signal peptide sequence, a varicella-zoster virus (VZV) secretion signal peptide sequence, a measles secretion signal peptide sequence, a rubella secretion signal peptide sequence, a mumps secretion signal peptide sequence, an Ebola secretion signal peptide sequence, a smallpox secretion signal peptide sequence, and a rabies secretion signal peptide sequence.

In certain embodiments, the viral secretion signal peptide is selected from the group consisting of: an influenza hemagglutinin (HA) secretion signal peptide sequence, a SARS CoV-2 spike secretion signal peptide sequence, a VZV gB secretion signal peptide sequence, a VZV gE secretion signal peptide sequence, a VZV gI secretion signal peptide sequence, a VZV gK secretion signal peptide sequence, a measles F-protein secretion signal peptide sequence, a rubella E1 protein secretion signal peptide sequence, a rubella E2 protein secretion signal peptide sequence, a mumps F-protein secretion signal peptide sequence, an Ebola GP protein secretion signal peptide sequence, a smallpox 6kDa IC protein secretion signal peptide sequence, and a rabies G protein secretion signal peptide sequence, preferably wherein the viral secretion signal peptide comprises an HA secretion signal peptide sequence from influenza A or influenza B, more preferably from influenza A.

In certain embodiments, the HA secretion signal peptide sequence comprises an amino acid sequence MKX1X2LX3VX4LX5TFX6X7X8X9A (SEQ ID NO: 856) wherein X1 is selected from A and V; X2 is selected from I and K; X3 is selected from V and L; X4 is selected from L and M; X5 is selected from Y and C; X6 is selected from T and A; X7 is selected from T and A; X8 is selected from A and T; and X9 is selected from N and Y.

In certain embodiments, the HA secretion signal peptide sequence comprises an amino acid sequence selected from MKAKLLVLLCTFTATYA (SEQ ID NO: 187), MKAILVVLLYTFTTANA (SEQ ID NO: 846), MKVKLLVLLCTFTATYA (SEQ ID NO: 847), MKAILVVLLYTFATANA (SEQ ID NO: 188) and MKAILVVMLYTFTTANA (SEQ ID NO: 848).

In certain embodiments, the HA secretion signal peptide sequence comprises an amino acid sequence MKX1IIALSX2ILCLVFX3 (SEQ ID NO: 857) wherein X1 is selected from T and A; X2 is selected from Y, N, C, and H; and X3 is selected from T and A.

In certain embodiments, the HA secretion signal peptide sequence comprises an amino acid sequence selected from MKTIIALSYILCLVFT (SEQ ID NO: 849), MKTIIALSYILCLVFA (SEQ ID NO: 193), MKTIIALSNILCLVFA (SEQ ID NO: 850), MKAIIALSNILCLVFA (SEQ ID NO: 851), MKTIIALSCILCLVFA (SEQ ID NO: 852) and MKTIIALSHILCLVFA (SEQ ID NO: 853).

In certain embodiments, the HA secretion signal peptide sequence comprises an amino acid sequence MKAIIVLLMVVTSX1A (SEQ ID NO: 858) wherein X1 is selected from S and N.

In certain embodiments, the HA secretion signal peptide sequence comprises an amino acid sequence MX1AIIVLLMVVTSNA (SEQ ID NO: 859) wherein X1 is selected from K and E.

In certain embodiments, the HA secretion signal peptide sequence comprises an amino acid sequence selected from MKAIIVLLMVVTSNA (SEQ ID NO: 194), MKAIIVLLMVVTSSA (SEQ ID NO: 854), and MEAIIVLLMVVTSNA (SEQ ID NO: 855).

In certain embodiments, the viral secretion signal peptide comprises an amino acid sequence selected from the group consisting of: MKAKLLVLLCTFTATYA (SEQ ID NO: 187); MKAILVVLLYTFATANA (SEQ ID NO: 188); MKTIIALSYILCLVFA (SEQ ID NO: 193); MKAIIVLLMVVTSNA (SEQ ID NO: 194); MFVFLVLLPLVS (SEQ ID NO: 195); MFLTTKRTMFVFLVLLPLVS (SEQ ID NO: 196); MSPCGYYSKWRNRDRPEYRRNLRFRFFSSIHNPAAAGSGFNGPGVFITSVTGVWLCFLCIFSMFVTAVVS (SEQ ID NO: 803); MGTVNKPVVGVLMGFGIITGTLRITNPVRA (SEQ ID NO: 804); MFLIQCLISAVIFYIQVTNA (SEQ ID NO: 805); MQALGIKTEHFIMCLLSGHA (SEQ ID NO: 806); MGLKVNVSIAFMAVLLTLQTPTG (SEQ ID NO: 807); MGAAAALTAVVLQGYNPPAYG (SEQ ID NO: 808); MGAPQAFLAGLLAAVAVGTARA (SEQ ID NO: 809); MKVFLVTCLGFAVFSSSVC

(SEQ ID NO: 810); MGV TGILQLPRDRFKRTSFFLWVILFQRTFS (SEQ ID NO: 811); MRS LIIFLLFPSIIYS (SEQ ID NO: 812); and MVPQALLFVPLL VFPLCFG (SEQ ID NO: 845).

In certain embodiments, the viral secretion signal peptide comprises an amino acid sequence of MKAKLLVLLCTFTATYA (SEQ ID NO: 187).

- 5 In certain embodiments, the viral secretion signal peptide is positioned at the N-terminus of the antigenic prokaryotic polypeptide. In certain embodiments, the viral secretion signal peptide is positioned at the N-terminus of the polypeptide disclosed herein.

- In certain embodiments, the viral secretion signal peptide is positioned at the C-terminus of the antigenic prokaryotic polypeptide. In certain embodiments, the viral secretion signal peptide is positioned at the C-terminus of the polypeptide disclosed herein.
- 10

In certain embodiments, the viral secretion signal peptide is attached to the antigenic prokaryotic polypeptide with a linker. In certain embodiments, the viral secretion signal peptide is attached to the polypeptide disclosed herein with a linker.

Heterologous transmembrane domains (TMBs)

- 15 The chimeric *Chlamydia sp.* MOMP VD polypeptide, *Chlamydia sp.* CT443 polypeptide, *Chlamydia sp.* CT584 polypeptide, *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide as described herein may comprise a heterologous transmembrane domain. The inclusion of a TMB may be advantageous as this will localise the antigen to the cell membrane. This may reduce antigen intracellular localisation and further promote higher immunogenicity relative to the antigen without the TMB sequence.
- 20 The chimeric *Chlamydia sp.* MOMP VD polypeptide, *Chlamydia sp.* CT443 polypeptide, *Chlamydia sp.* CT584 polypeptide, *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide comprising a heterologous transmembrane domain may also comprise a secretion signal peptide sequence. Typically, a nucleic acid described herein encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a heterologous transmembrane domain also comprises a nucleotide sequence encoding a
- 25 secretion signal peptide sequence. Typically, a nucleic acid described herein encoding the *Chlamydia sp.* CT443 polypeptide that comprises a heterologous transmembrane domain also comprises a nucleotide sequence encoding a secretion signal peptide sequence. Typically, a nucleic acid described herein encoding the *Chlamydia sp.* CT584 polypeptide that comprises a heterologous transmembrane domain also comprises a nucleotide sequence encoding a secretion signal peptide sequence. Typically, a nucleic acid described
- 30 herein encoding the *Chlamydia sp.* CT600 polypeptide that comprises a heterologous transmembrane domain also comprises a nucleotide sequence encoding a secretion signal peptide sequence. Typically, a nucleic acid described herein encoding the *Chlamydia sp.* CT812 polypeptide that comprises a heterologous transmembrane domain also comprises a nucleotide sequence encoding a secretion signal peptide sequence.

The TMB may be from any known TMB in the art, including but not limited to, TMBs from eukaryotic transmembrane proteins (e.g., mammalian transmembrane proteins, such as human transmembrane proteins), TMBs from prokaryotic transmembrane proteins, and TMBs from viral transmembrane proteins. TMBs may further be identified through in silico prediction algorithms, for example, in the TMHMM prediction method described in Krogh et al. (J Mol Biol. 305(3): 567-580. 2001) and <https://services.healthtech.dtu.dk/services/TMHMM-2.0/>, each of which is incorporated herein by reference in their entirety. Some features of TMBs are described in further detail in Albers et al. (Chapter 2 – cell membrane structures and functions. Basic Neurochemistry eighth edition. Pages 26-39. 2012), incorporated herein by reference. TMBs are typically, but not exclusively, comprised predominantly of nonpolar (hydrophobic) amino acid residues and may traverse a lipid bilayer once or several times. The skilled person knows well methods to determine the hydrophobicity of an amino acid. See Simm et al. (2016), Biol Res., 49(1):31; Wimlet and White (1996), Nat Struct Biol., 3(10): 842-848; https://blanco.biomol.uci.edu/hydrophobicity_scales.html; and <https://www.cgl.ucsf.edu/chimera/docs/UsersGuide/midas/hydrophob.html>.

The TMBs usually comprise alpha helices, each helix containing 18-21 amino acids, which is sufficient to span the lipid bilayer. Accordingly, in certain embodiments, the transmembrane domain comprises one or more alpha helices. In some embodiments, the TMB: (a) comprises or consists of 15 to 50 amino acid residues, preferably 15 to 30 amino acid residues, more preferably 18 to 25 amino acid residues ; and/or (b) comprises at least 50%, at least 55%, or at least 60% of hydrophobic amino acid residues, preferably selected in the group consisting of: alanine, isoleucine, leucine, valine, phenylalanine, tryptophane and tyrosine; and/or (c) comprises at least one alpha helix.

In certain embodiments, the transmembrane domain is derived from an integral membrane protein, as further defined hereafter and in Albers et al., An “integral membrane protein” (also known as an intrinsic membrane protein) is a membrane protein that is permanently attached to the lipid membrane. In certain embodiments, the transmembrane domain is derived from an integral polytopic protein. An integral polytopic protein is one that spans the entire membrane. In certain embodiments, the transmembrane domain is derived from a single pass (trans)membrane protein, more particularly a bitopic membrane protein, e.g., of Type I or Type II. Single-pass membrane proteins cross the membrane only once (i.e., a bitopic membrane protein), while multi-pass membrane proteins weave in and out, crossing several times. Single pass transmembrane proteins can be categorized as Type I, which are positioned such that their carboxyl-terminus is towards the cytosol, or Type II, which have their amino-terminus towards the cytosol. In certain embodiments, the transmembrane domain is derived from an integral monotopic protein. An integral monotopic protein is one that is associated with the membrane from only one side and does not span the lipid bilayer completely.

In certain embodiments, the heterologous transmembrane domain is derived from a non-human sequence.

In certain embodiments, the heterologous transmembrane domain is derived from a viral sequence. The phrase “influenza”, “SARS CoV-2”, “varicella-zoster virus (VZV)”, “measles”, “rubella”, “rabies,” “Ebola,” and “smallpox” preceding the phrase “transmembrane domain sequence” indicates that the transmembrane domain sequence was derived from the virus corresponding to that name.

- 5 In certain embodiments, the heterologous transmembrane domain is derived from a viral transmembrane domain sequence selected from the group consisting of: an influenza transmembrane domain sequence, a SARS CoV-2 transmembrane domain sequence, a varicella-zoster virus (VZV) transmembrane domain sequence, a measles transmembrane domain sequence, a rubella transmembrane domain sequence, a mumps transmembrane domain sequence, a rabies transmembrane domain sequence, and an Ebola transmembrane domain sequence. These particular transmembrane domains are derived from viral sequences in viruses which have been administered to humans as vaccines (live-attenuated, inactivated or mRNA), with demonstrated strong safety profiles.

- 15 In certain embodiments, the heterologous transmembrane domain is selected from the group consisting of: an influenza hemagglutinin (HA) transmembrane domain sequence, a SARS CoV-2 spike transmembrane domain sequence, a VZV gB transmembrane domain sequence, a VZV gE transmembrane domain sequence, a VZV gI transmembrane domain sequence, a VZV gK transmembrane domain sequence, a measles F-protein transmembrane domain sequence, a rubella E1 protein transmembrane domain sequence, a rubella E2 protein transmembrane domain sequence, a mumps F-protein transmembrane domain sequence, a rabies virus glycoprotein (Rabies G) transmembrane domain sequence, and an Ebola GP protein transmembrane domain sequence.

In certain embodiments, the heterologous transmembrane domain comprises an HA transmembrane domain sequence from influenza A or influenza B, preferably from influenza A.

Exemplary viral transmembrane domain amino acid sequences of the disclosure are shown below in Table 3.

25 **Table 3 – Viral Transmembrane Domain (TMB) Signal Amino Acid Sequences**

NAME OF THE PROTEIN	ORGANISM	STRAIN	SEQUENCE OF THE TMB
HA (H1N1)	Influenza virus	A/New Caledonia/20/1999	ILAIYSTVASSLVLLVSLGAISF (SEQ ID NO: 813)
HA (H1N1pdm)	Influenza virus	A/California/7/2009	ILAIYSTVASSLVLVVSLGAISF (SEQ ID NO: 814)
HA (H3N2)	Influenza virus	A/Moscow/10/1999	ILWISFAISCFLLCVVLLGFI (SEQ ID NO: 815)
HA B	Influenza virus	B/Phuket/3073/2013	STAASSLAVTLM LAIFIVYMV (SEQ ID NO: 816)
Spike	SARS CoV-2	Wuhan-1	WYIWLGFIAGLIAIVMVTIML (SEQ ID NO: 817)
gB	VZV	Oka strain	FGALAVGLLVLAGLVAAFFAY

			(SEQ ID NO: 818)
gE	VZV	Oka strain	AAWTGGLAAVVLLCLVIFLIC (SEQ ID NO: 819)
gI	VZV	Oka strain	IIPIVASVMILTAMVIVIVI (SEQ ID NO: 820)
gK	VZV	Oka strain	YFWCVQLKMIFFAWFVYGYML (SEQ ID NO: 821)
F	Measles	Edmonston-Zagreb strain	IVYILIAVCLGGLIGIPALIC (SEQ ID NO: 822)
E1	Rubella	RA27/3 strain	LDHAFAAFVLLVPWVLIFMVC (SEQ ID NO: 823)
E2	Rubella	RA27/3 strain	WWQLTLGAICALLAGLLACC (SEQ ID NO: 824)
F	Mumps	Miyahara strain	IVAALVLSILSIHSLLFCCW (SEQ ID NO: 825)
GP	Ebola	Mayinga-76 strain	WIPAGIGVTGVIIAVIALFCI (SEQ ID NO: 826)
Rabies G	Rabies	Rabies Pasteur strain	VLLSAGALTALMLIIFLMTWCW (SEQ ID NO: 860)

In certain embodiments, the heterologous TMB sequence is positioned at the N-terminus or the C-terminus (e.g., C-terminus) of a polypeptide described herein.

In certain embodiments, the TMB amino acid sequence is encoded by a codon-optimized polynucleotide sequence.

In some embodiments, the chimeric MOMP VD polypeptide comprises a heterologous transmembrane domain as described herein. In some embodiments, the heterologous transmembrane domain does not comprise the sequence of SEQ ID NO: 189. In some embodiments, the heterologous transmembrane domain comprises the sequence of SEQ ID NO: 190. Typically, the chimeric MOMP VD polypeptide does not comprise a heterologous transmembrane domain.

In alternative embodiments, one or more of the chimeric MOMP VD polypeptide, the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide and/or *Chlamydia sp.* CT812 polypeptide as described herein do not comprise a heterologous transmembrane domain. Such polypeptides may comprise a secretion signal peptide sequence. In further embodiments, the polypeptides of the invention are secreted. In some embodiments, the chimeric MOMP VD polypeptide described herein is a secreted polypeptide. In some embodiments, the *Chlamydia sp.* CT443 polypeptide described herein is a secreted polypeptide. In some embodiments, the *Chlamydia sp.* CT584 polypeptide described herein is a secreted polypeptide. In some embodiments, the *Chlamydia sp.* CT600 polypeptide described herein is a secreted polypeptide. In some embodiments, the *Chlamydia sp.* CT812 polypeptide described herein is a secreted polypeptide. In preferred embodiments, the modified MOMP polypeptide described herein is a secreted polypeptide. Secreted polypeptides described herein comprise a secretion signal peptide sequence.

In certain embodiments the TMB: (a) comprises or consists of 15 to 50 amino acid residues, preferably 15 to 30 amino acid residues, more preferably 18 to 25 amino acid residues; and/or (b) comprises at least 50% of hydrophobic amino acid residues, preferably selected in the group consisting of: alanine, isoleucine, leucine, valine, phenylalanine, tryptophane and tyrosine; and/or (c) comprises at least one alpha helix.

- 5 In certain embodiments the TMB is derived from an integral membrane protein, preferably from a single pass membrane protein, more preferably from a bitopic membrane protein, even more preferably from a bitopic membrane protein of Type I).

In certain embodiments the TMB is derived from a non-human sequence.

In certain embodiments, the TMB is derived from a viral sequence.

- 10 In certain embodiments, the TMB is derived from a viral transmembrane domain sequence selected from the group consisting of: an influenza transmembrane domain sequence, and a non-influenza transmembrane domain sequence selected from the group consisting of a SARS CoV-2 transmembrane domain sequence, a varicella-zoster virus (VZV) transmembrane domain sequence, a measles transmembrane domain sequence, a rubella transmembrane domain sequence, a mumps transmembrane domain sequence, an Ebola
15 transmembrane domain sequence, and a rabies transmembrane domain sequence.

- In certain embodiments, the TMB is selected from the group consisting of: an influenza hemagglutinin (HA) transmembrane domain sequence, a SARS CoV-2 spike transmembrane domain sequence, a VZV gB transmembrane domain sequence, a VZV gE transmembrane domain sequence, a VZV gI transmembrane domain sequence, a VZV gK transmembrane domain sequence, a measles F-protein transmembrane domain
20 sequence, a rubella E1 protein transmembrane domain sequence, a rubella E2 protein transmembrane domain sequence, a mumps F-protein transmembrane domain sequence, an Ebola GP protein transmembrane domain sequence, and a rabies G protein transmembrane domain sequence, preferably wherein the TMB comprises an HA transmembrane domain sequence from influenza A or influenza B, more preferably from influenza A.

- 25 In certain embodiments, the TMB comprises an amino acid sequence selected from the group consisting of: ILAIYSTVASSLVLLVSLGAISF (SEQ ID NO: 813); ILAIYSTVASSLVLVVSLGAISF (SEQ ID NO: 814); ILWISFAISCFLLCVLLGFI (SEQ ID NO: 815); STAASSLAVTLMIAIFIVYMV (SEQ ID NO: 816); WYIWLGFIAGLIAIVMVTIML (SEQ ID NO: 817); FGALAVGLLVLAGLVAAFFAY (SEQ ID NO: 818); AAWTGGLAAVLLCLVIFLIC (SEQ ID NO: 819); IIPIVASVMILTAMVIVIVI (SEQ
30 ID NO: 820); YFWCVQLKMIFFAWFVYGYMYL (SEQ ID NO: 821); IVYILIAVCLGGLIGIPALIC (SEQ ID NO: 822); LDHAFAAFVLLVPWVLIFMVC (SEQ ID NO: 823); WWQLTLGAICALLLAGLLACC (SEQ ID NO: 824); IVAALVLSILSIISLLFCCW (SEQ ID NO: 825); WIPAGIGVTGVIIAVALFCI (SEQ ID NO: 826); and VLLSAGALTALMLIIFLMTCW (SEQ ID NO: 860).

In certain embodiments, the TMB comprises an amino acid sequence of ILAIYSTVASSLVLLVSLGAISF (SEQ ID NO: 813).

In certain embodiments, the TMB is attached to a polypeptide described herein with a linker.

In certain embodiments, the TMB is positioned at the N-terminus of a polypeptide described herein.

- 5 In certain embodiments, the TMB is positioned at the C-terminus of a polypeptide described herein.

Linkers

In certain embodiments of the disclosure, the secretion signal peptide (SS) sequence or transmembrane domain (TMB) are directly fused to a polypeptide described herein (i.e., there is no linker, such as an amino acid linker, connecting the SS sequence or TMB to the polypeptide described herein).

- 10 In other embodiments, the SS sequences and TMBs of the disclosure are optionally attached to a polypeptide described herein with a linker. In certain embodiments, the linker is an amino acid linker. In the certain embodiments, the amino acid linker is 1-10 amino acids in length (e.g., the amino acid linker has a length of 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, or 10 amino acids).

- 15 Illustrative examples of linkers include glycine polymers (Gly)_n, where n is an integer of at least one, two, three, four, five, six, seven, or eight; glycine-serine polymers (GlySer)_n, where n is an integer of at least one, two, three, four, five, six, seven, or eight; glycine-alanine polymers; alanine-serine polymers; and other flexible linkers known in the art.

- Glycine and glycine-serine polymers are relatively unstructured and flexible, and therefore may be able to
20 serve as a neutral tether between the SS sequence and/or TMB and the polypeptides described herein. In certain embodiments, the linker is SGS or GSG.

- Other exemplary linkers include, but are not limited to, the following amino acid sequences: GGG; DGGGS (SEQ ID NO: 827); TGEKP (SEQ ID NO: 828) (Liu et al. Proc. Natl. Acad. Sci. 94: 5525-5530. 1997); GGRR (SEQ ID NO: 829); (GGGS)_n (SEQ ID NO: 830), wherein n = 1, 2, 3, 4 or 5 (Kim et al. Proc.
25 Natl. Acad. Sci. 93: 1156-1160. 1996); EGKSSGSGSESKVD (SEQ ID NO: 831) (Chaudhary et al. Proc. Natl. Acad. Sci. 87: 1066-1070. 1990); KESGSVSSEQLAQFRSLD (SEQ ID NO: 832) (Bird et al. Science. 242:423-426. 1988), GGRRGGGS (SEQ ID NO: 833); LRQRDGERP (SEQ ID NO: 834); LRQKDGGGSERP (SEQ ID NO: 835); and GSTSGSGKPGSGEGSTKG (SEQ ID NO: 836) (Cooper et al. Blood. 101(4): 1637-1644. 2003). Preferred linkers are shorter, e.g., consisting of 3, 4 or 5 amino acids.

- 30 Additional examples of linkers are provided in Chen et al. (Adv Drug Deliv Rev. 65(10): 1357-1369. 2013), incorporated herein by reference.

Compositions

The invention provides a composition comprising one or more nucleic acids of the disclosure. The invention also provides a composition comprising one or more polypeptides of the disclosure. A composition of the invention may be a pharmaceutical composition, e.g., comprising a pharmaceutically acceptable carrier, excipient or diluent. In certain embodiments, the composition of the invention is an immunogenic composition. An “immunogenic composition” means a composition comprising a nucleic acid or protein that, when administered to a subject, elicits an immune response, e.g., an antigen-specific immune response. The response may be a T cell immune response or an antibody response. The composition of the invention may be a vaccine composition. Immunogenic compositions (e.g., vaccine compositions) may elicit protective immunity (e.g., T cell and/or B cell (i.e. antibody) responses) against *Chlamydia sp.* infection. The T cell response may include a CD4+ T cell response and/or a CD8+ T cell response, such as IFN γ -producing CD4+ T cells and/or IFN γ -producing CD8+ T cells. In some embodiments, the T cell response includes CD4+ T cells, such as IFN γ -producing CD4+ T cells.

“Protective immunity” or a “protective immune response”, as used herein, refers to immunity or eliciting an immune response against an infectious agent (e.g., a Chlamydia pathogen), which is exhibited by a subject, that prevents or ameliorates an infection or reduces at least one symptom thereof. Specifically, induction of protective immunity or a protective immune response from administration of a composition of the invention is evident by elimination or reduction of the presence of one or more symptoms of the Chlamydia infection and/or an expansion of the Chlamydia-responsive memory T cell population. As used herein, the term “immune response” refers to both the humoral immune response and the cell-mediated immune response. Preferably, the protective immunity provided by the invention is characterised by protective immunological memory (e.g., a protective memory T cell response) against the pathogen. The protective immunity may be characterised by an effective pathogen-responsive (e.g., Chlamydia-responsive) memory T cell population. In preferred embodiments, treatment with a composition of the invention as described herein provides protective immunity against re-infection by the Chlamydia pathogen. Protective immunity may be sterilising immunity (i.e. complete protective immunity), whereby the protected subject can elicit an immune response which completely eliminates the infection. Chlamydia antigens described herein (e.g., the chimeric MOMP VD polypeptides of the invention and the *Chlamydia sp.* CT443 and CT584 polypeptides of the invention) may bind *Chlamydia sp.* (e.g., *C. trachomatis*) elementary bodies, e.g., elementary bodies of two or more serotypes.

Nucleic acid compositions

In one aspect the invention provides a composition comprising a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein.

In another aspect, the invention provides a composition comprising a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein.

In another aspect, the invention provides a composition comprising a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein.

5 In another aspect, the invention provides a composition comprising a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein.

In another aspect, the invention provides a composition comprising a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide as described herein.

10 In another aspect, the invention provides a composition comprising a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide as described herein.

In a further aspect, the invention provides a composition comprising one, two, three or four of: (a) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein; (b) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein; (c) a nucleic acid as described herein that comprises
15 a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide as described herein; and/or (d) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide as described herein.

A composition of the invention may comprise a combination as described herein of the nucleic acids as described herein (e.g., they may be formulated in the same composition). The combinations as described
20 herein of the nucleic acids as described herein may alternatively be in two or more separate compositions (e.g., as a combination of compositions for simultaneous, separate or sequential administration, (e.g., in a therapeutic use as described herein)).

In some embodiments, the composition may comprise (a) a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein, (b) a nucleic acid as
25 described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein, and (c) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein.

In some embodiments, the composition may comprise (a) a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein, (b) a nucleic acid as
30 described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein, and (c) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein.

Typically, the composition may comprise (a) a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein, (b) a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein, (c) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein, and (d) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein.

A composition of the present disclosure comprising one or more nucleic acids of the present disclosure can also include one or more additional components such as small molecule immunopotentiators (e.g., TLR agonists). A composition of the present disclosure can also include a delivery system for a nucleic acid described herein (e.g., RNA), such as a liposome, an oil-in-water emulsion, or a microparticle. In some embodiments, the composition comprises a lipid nanoparticle (LNP). In certain embodiments, the composition comprises a nucleic acid molecule of the invention encapsulated within an LNP.

Polypeptide compositions

In one aspect the invention provides a composition comprising a modified MOMP polypeptide as described herein.

In another aspect, the invention provides a composition comprising a chimeric MOMP VD polypeptide as described herein.

In another aspect, the invention provides a composition comprising a *Chlamydia sp.* CT443 polypeptide as described herein.

In another aspect, the invention provides a composition comprising a *Chlamydia sp.* CT584 polypeptide as described herein.

In another aspect, the invention provides a composition comprising a *Chlamydia sp.* CT600 polypeptide as described herein.

In another aspect, the invention provides a composition comprising a *Chlamydia sp.* CT812 polypeptide as described herein.

In a further aspect, the invention provides a composition comprising one, two, three or four of: (a) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT443 polypeptide as described herein; (b) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT584 polypeptide as described herein; (c) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT600 polypeptide as described herein; and/or (d) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT812 polypeptide as described herein.

A composition of the invention may comprise a combination as described herein of the polypeptides as described herein (e.g., they may be formulated in the same composition). The combinations as described herein of the polypeptides as described herein may alternatively be in two or more separate compositions (e.g., as a combination of compositions for simultaneous, separate or sequential administration, (e.g., in a therapeutic use as described herein)).

In some embodiments, the composition may comprise (i) a modified MOMP polypeptide as described herein, (ii) a chimeric MOMP VD polypeptide as described herein, and (iii) a *Chlamydia sp.* CT443 polypeptide as described herein.

In some embodiments, the composition may comprise (i) a modified MOMP polypeptide as described herein, (ii) a chimeric MOMP VD polypeptide as described herein, and (iii) a *Chlamydia sp.* CT584 polypeptide as described herein.

Typically, the composition may comprise (i) a modified MOMP polypeptide as described herein, (ii) a chimeric MOMP VD polypeptide as described herein, (iii) a *Chlamydia sp.* CT443 polypeptide as described herein, and (iv) a *Chlamydia sp.* CT584 polypeptide as described herein.

A composition of the present disclosure comprising one or more polypeptides of the present disclosure may comprise an adjuvant. As used herein, an “adjuvant” refers to a substance or vehicle that enhances the immune response to an antigen. Adjuvants can include, without limitation, a suspension of minerals (e.g., alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; a water-in-oil or oil-in-water emulsion in which antigen solution is emulsified in mineral oil or in water (e.g., Freund’s incomplete adjuvant). Sometimes killed mycobacteria is included (e.g., Freund’s complete adjuvant) to further enhance antigenicity. Adjuvants can include squalene based oil in water emulsion adjuvants (e.g., AF03 e.g as described in WO2007006939 and U.S. Patent No. 8,703,095; AS03, e.g., as described in WO1995017209, WO1995017210 and U.S. Patent Nos. 6,623,739, 7,029,678 and 7,510,698; and MF59, e.g as described in WO1990014837 and U.S. Patent Nos. 6,299,884 and 6,451,325). Immuno-stimulatory oligonucleotides (e.g., a CpG motif) can also be used as adjuvants (for example, see U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; 6,339,068; 6,406,705; and 6,429,199). Adjuvants can also include biological molecules, such as Toll-Like Receptor (TLR) agonists (e.g., AS01, e.g., as described in WO2007068907 and U.S. Patent Nos. 10,039,823 and 10,143,745; SPA14, e.g., as described in WO2022090359; and LEQ, e.g., as described in WO2023056089) and costimulatory molecules. In some embodiments, the adjuvant is AF03 (an oil-in-water squalene-based emulsion adjuvant). In some embodiments, the adjuvant is one that can elicit a Th1 response.

Nucleic acid combinations

The invention provides combinations comprising two or more nucleic acids of the invention.

Thus, in one aspect the invention provides a combination comprising a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein and:

(i) a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein; and /or

5 (ii) one or more of

(a) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein;

(b) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein;

10 (c) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide as described herein; and/or

(d) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide as described herein.

15 In some embodiments, the combination comprises the nucleic acid of (a), the nucleic acid of (b), the nucleic acid of (c), the nucleic acid of (d), the nucleic acids of (a) and (b), the nucleic acids of (a) and (c), the nucleic acids of (a) and (d), the nucleic acids of (b) and (c), the nucleic acids of (b) and (d), the nucleic acids of (c) and (d), the nucleic acids of (a), (b) and (c), the nucleic acids of (a), (c) and (d), the nucleic acids of (a), (b) and (d), the nucleic acids of (b), (c) and (d), or the nucleic acids of (a), (b), (c) and (d). In some embodiments, the combination comprises the nucleic acid of (a). In some embodiments, the
20 combination comprises the nucleic acid of (b). Typically, the combination comprises the nucleic acid of (a) and the nucleic acid of (b).

The nucleic acids of any of (a)-(d) may be located on the same nucleic acid molecule. Alternatively, the nucleic acids of (a)-(d) may be separate nucleic acid molecules.

25 Thus, in some embodiments, a combination of the invention comprises a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein and a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein.

30 In some embodiments, a combination of the invention comprises a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein and one, two, three or four of (a) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein; (b) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein; (c) a

nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide as described herein; and/or (d) a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide as described herein. The combination may comprise any of the (a)-(d) non-MOMP antigen combinations set out above. In some embodiments, the combination comprises the nucleic acid of (a). In some embodiments, the combination comprises the nucleic acid of (b). Typically, the combination comprises the nucleic acid of (a) and the nucleic acid of (b).

In some embodiments, a combination of the invention comprises (i) a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein; (ii) a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein; and (iii) one, two, three or four of the nucleic acids in (a)-(d). The combination may comprise any of the (a)-(d) non-MOMP antigen combinations set out above. In some embodiments, the combination comprises the nucleic acid of (a). In some embodiments, the combination comprises the nucleic acid of (b). Typically, the combination comprises the nucleic acid of (a) and the nucleic acid of (b).

In a further aspect, the invention provides a combination comprising a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein and one, two, three or four of the nucleic acids in (a)-(d). The combination may comprise any of the (a)-(d) non-MOMP antigen combinations set out above. In some embodiments, the combination comprises the nucleic acid of (a). In some embodiments, the combination comprises the nucleic acid of (b). Typically, the combination comprises the nucleic acid of (a) and the nucleic acid of (b). In some embodiments, the combination further comprises a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein.

In another aspect, the invention provides a combination comprising a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein and one, two or three of the nucleic acids as defined in (b)-(d). The combination may comprise any of the non-MOMP antigen combinations of (b), (c) and/or (d) set out above. Typically, the combination comprises the nucleic acid of (b). In some embodiments, the combination further comprises a nucleic acid comprising a nucleotide sequence encoding a native *Chlamydia sp.* MOMP polypeptide or a variant thereof.

In another aspect, the invention provides a combination comprising a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein and one, two or three of the nucleic acids as defined in (a), (c) or (d). The combination may comprise any of the non-MOMP antigen combinations of (a), (c) and/or (d) set out above. Typically, the combination comprises the nucleic acid of (a). In some embodiments, the combination further comprises a nucleic acid comprising a nucleotide sequence encoding a native *Chlamydia sp.* MOMP polypeptide or a variant thereof.

In another aspect, the invention provides a combination comprising a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide as described herein and one, two or three of the nucleic acids as defined in (a), (b) or (d). The combination may comprise any of the non-MOMP antigen combinations of (a), (b) and/or (d) set out above. In some embodiments, the combination further comprises a nucleic acid comprising a nucleotide sequence encoding a native *Chlamydia sp.* MOMP polypeptide or a variant thereof.

In another aspect, the invention provides a combination comprising a nucleic acid as described herein that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide as described herein and one, two or three of the nucleic acids as defined in (a)-(c). The combination may comprise any of the non-MOMP antigen combinations of (a), (b) and/or (c) set out above. In some embodiments, the combination further comprises a nucleic acid comprising a nucleotide sequence encoding a native *Chlamydia sp.* MOMP polypeptide or a variant thereof.

Thus, the combination of the invention may comprise a nucleic acid comprising a nucleotide sequence encoding a native *Chlamydia sp.* MOMP polypeptide, or a variant thereof, and one, two, three or four of the nucleic acids in (a)-(d). Variants of a native *Chlamydia sp.* MOMP polypeptide include MOMP polypeptides comprising one or more conservative amino acid substitutions, MOMP polypeptides comprising a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to a cysteine residue in a native *Chlamydia sp.* MOMP polypeptide and MOMP polypeptides comprising a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to a glycosylation site, preferably an N-glycosylation site, in a native *Chlamydia sp.* MOMP polypeptide. Variants of a native *Chlamydia sp.* MOMP polypeptide also include MOMP polypeptides that comprise a secretion signal peptide sequence and MOMP polypeptides comprising a heterologous transmembrane domain. In some embodiments, a variant of a native *Chlamydia sp.* MOMP polypeptide comprises a secretion signal peptide sequence. In some embodiments, a variant of a native *Chlamydia sp.* MOMP polypeptide comprises a secretion signal peptide sequence and a heterologous transmembrane domain.

The combinations of the nucleic acids as disclosed herein encompass all of the individual nucleic acids being in the same composition and all of the individual nucleic acids being in one or more separate compositions. In some embodiments, all of the individual nucleic acids in a combination as described herein are in the same composition. In some embodiments, each of the nucleic acids in a combination as described herein is a separate composition. In some embodiments, two or more of the nucleic acids in a combination as described herein are in two or more compositions. In some embodiments of the combinations of the invention, the one, two, three or four of the nucleic acids of (a)-(d) above are in one or more compositions. In some embodiments of the combinations of the invention, the two, three or four of the nucleic acids of (a)-(d) above are in two or more separate compositions. Typically, all of the individual nucleic acids are in the same composition.

Polypeptide combinations

The invention provides combinations comprising two or more polypeptides of the invention.

Thus, in one aspect, the invention provides a combination comprising a modified MOMP polypeptide as described herein and:

- 5 (i) a chimeric MOMP VD polypeptide as described herein; and/or
- (ii) one or more of
- (a) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT443 polypeptide as described herein;
- (b) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT584 polypeptide as described herein:
- 10 (c) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT600 polypeptide as described herein; and/or
- (d) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT812 polypeptide as described herein.

- 15 In some embodiments, the combination comprises the polypeptide of (a), the polypeptide of (b), the polypeptide of (c), the polypeptide of (d), the polypeptides of (a) and (b), the polypeptides of (a) and (c), the polypeptides of (a) and (d), the polypeptides of (b) and (c), the polypeptides of (b) and (d), the polypeptides of (c) and (d), the polypeptides of (a), (b) and (c), the polypeptides of (a), (c) and (d), the polypeptides of (a), (b) and (d), the polypeptides of (b), (c) and (d), the polypeptides of (a), (b), (c) and (d).
- 20 In some embodiments, the combination comprises the polypeptide of (a). In some embodiments, the combination comprises the polypeptide of (b). Typically, the combination comprises the polypeptide of (a) and the polypeptide of (b).

Thus, in some embodiments, a combination of the invention comprises a modified MOMP polypeptide as described herein and a chimeric MOMP VD polypeptide as described herein.

- 25 In some embodiments, a combination of the invention comprises a modified MOMP polypeptide as described herein and one, two, three or four of (a) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT443 polypeptide as described herein; (b) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT584 polypeptide as described herein; (c) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT600 polypeptide as described herein; and/or (d) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT812 polypeptide as described herein. The combination may comprise any of the (a)-(d) non-MOMP antigen combinations set out above. In some
- 30 In some embodiments, the combination comprises the polypeptide of (a). In some embodiments, the combination

comprises the polypeptide of (b). Typically, the combination comprises the polypeptide of (a) and the polypeptide of (b).

In some embodiments, a combination of the invention comprises (i) a modified MOMP polypeptide as described herein; (ii) a chimeric MOMP VD polypeptide as described herein and (iii) one, two, three or four of the polypeptides in (a)-(d). The combination may comprise any of the (a)-(d) non-MOMP antigen combinations set out above. In some embodiments, the combination comprises the polypeptide of (a). In some embodiments, the combination comprises the polypeptide of (b). Typically, the combination comprises the polypeptide of (a) and the polypeptide of (b).

In a further aspect, the invention provides a combination comprising a chimeric MOMP VD polypeptide as described herein and one, two, three or four of the polypeptides in (a)-(d). The combination may comprise any of the (a)-(d) non-MOMP antigen combinations set out above. In some embodiments, the combination comprises the polypeptide of (a). In some embodiments, the combination comprises the polypeptide of (b). Typically, the combination comprises the polypeptide of (a) and the polypeptide of (b). In some embodiments, the composition further comprises a modified MOMP polypeptide as described herein.

In another aspect, the invention provides a combination comprising a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT443 polypeptide as described herein and one, two or three of the polypeptides as defined in (b)-(d). The combination may comprise any of the non-MOMP antigen combinations of (b), (c) and/or (d) set out above. Typically, the combination comprises the polypeptide of (b). In some embodiments, the combination further comprises a native *Chlamydia sp.* MOMP polypeptide or a variant thereof.

In another aspect, the invention provides a combination comprising a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT584 polypeptide as described herein and one, two or three of the polypeptides as defined in (a), (c) or (d). The combination may comprise any of the non-MOMP antigen combinations of (a), (c) and/or (d) set out above. Typically, the combination comprises the polypeptide of (a). In some embodiments, the combination further comprises a native *Chlamydia sp.* MOMP polypeptide or a variant thereof.

In another aspect, the invention provides a combination comprising a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT600 polypeptide as described herein and one, two or three of the polypeptides as defined in (a), (b) or (d). The combination may comprise any of the non-MOMP antigen combinations of (a), (b) and/or (d) set out above. In some embodiments, the combination further comprises a native *Chlamydia sp.* MOMP polypeptide or a variant thereof.

In another aspect, the invention provides a combination comprising a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT812 polypeptide as described herein and one, two or three of the polypeptides as defined in (a)-(c). The combination may comprise any of the non-MOMP antigen

combinations of (a), (b) and/or (c) set out above. In some embodiments, the combination further comprises a native *Chlamydia sp.* MOMP polypeptide or a variant thereof.

Thus, a combination of the invention may comprise a native *Chlamydia sp.* MOMP polypeptide, or a variant thereof, and one, two, three or four of the polypeptides in (a)-(d). Variants of a native *Chlamydia sp.* MOMP polypeptide include MOMP polypeptides comprising one or more conservative amino acid substitutions, MOMP polypeptides comprising a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to a cysteine residue in a native *Chlamydia sp.* MOMP polypeptide and MOMP polypeptides comprising a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to a glycosylation site, preferably an N-glycosylation site, in a native *Chlamydia sp.* MOMP polypeptide. Variants of a native *Chlamydia sp.* MOMP polypeptide also include MOMP polypeptides that comprise a secretion signal peptide sequence and MOMP polypeptides comprising a heterologous transmembrane domain. In some embodiments, a variant of a native *Chlamydia sp.* MOMP polypeptide comprises a secretion signal peptide sequence. In some embodiments, a variant of a native *Chlamydia sp.* MOMP polypeptide comprises a secretion signal peptide sequence and a heterologous transmembrane domain.

The combinations of the polypeptides as disclosed herein encompass all of the individual polypeptides being in the same composition and all of the individual polypeptides being in one or more separate compositions. In some embodiments, all of the individual polypeptides in a combination described herein are in the same composition. In some embodiments, each of the polypeptides in a combination as described herein is in a separate composition. In some embodiments, two or more of the polypeptides in a combination as described herein are in two or more compositions. In some embodiments of the combinations of the invention, the one, two, three or four of the polypeptides of (a)-(d) above are in one or more compositions. In some embodiments of the combinations of the invention, the two, three or four of the polypeptides of (a)-(d) above are in two or more separate compositions. Typically, all of the individual polypeptides are in the same composition.

LNPs

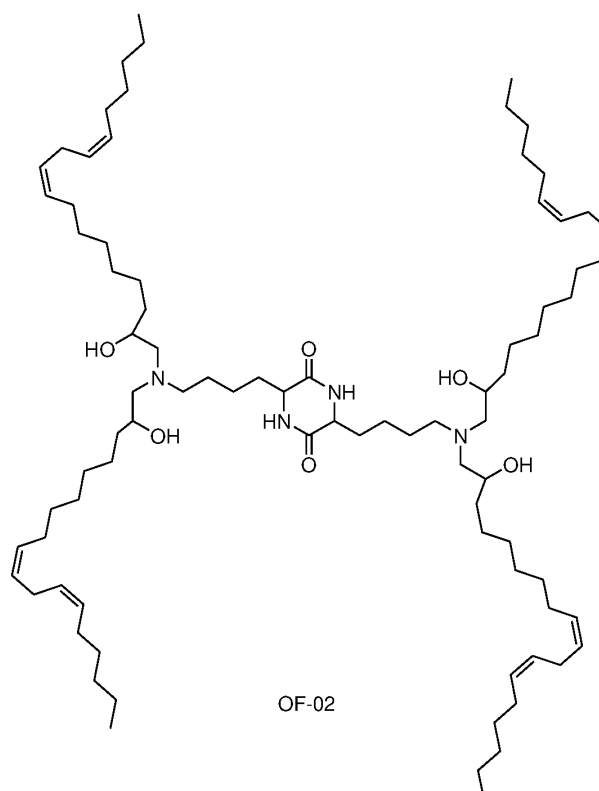
In certain embodiments, the composition of the invention (*e.g.*, the composition comprising a nucleic acid of the invention) further comprises a lipid nanoparticle (LNP). In certain embodiments, the nucleic acid of the invention is encapsulated in the LNP.

The LNPs of the disclosure may comprise four categories of lipids: (i) an ionizable lipid (*e.g.*, a cationic lipid); (ii) a PEGylated lipid; (iii) a cholesterol-based lipid, and (iv) a helper lipid.

A. Ionizable Lipids

An ionizable lipid facilitates mRNA encapsulation and may be a cationic lipid. A cationic lipid affords a positively charged environment at low pH to facilitate efficient encapsulation of the negatively charged mRNA drug substance.

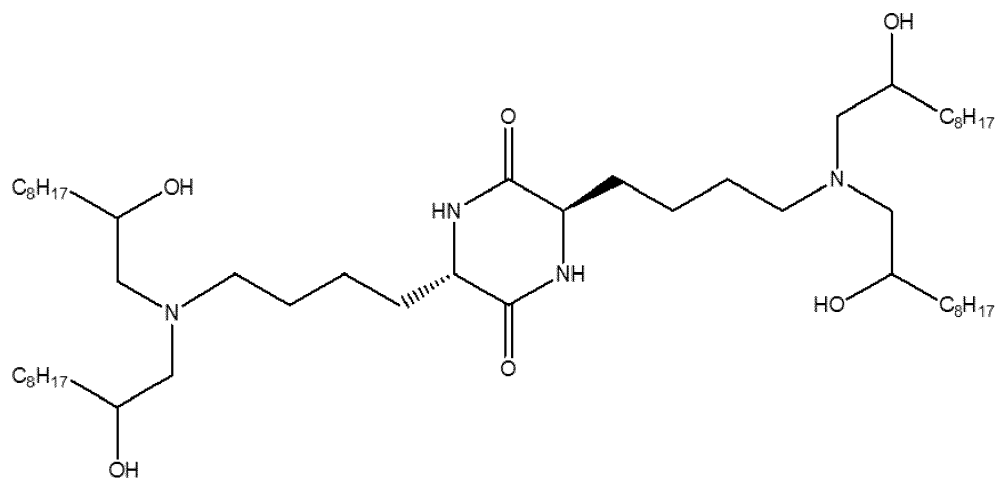
- 5 In some embodiments, the cationic lipid is OF-02:



Formula (I)

- 10 OF-02 is a non-degradable structural analog of OF-Deg-Lin. OF-Deg-Lin contains degradable ester linkages to attach the diketopiperazine core and the doubly-unsaturated tails, whereas OF-02 contains non-degradable 1,2-amino-alcohol linkages to attach the same diketopiperazine core and the doubly-unsaturated tails (Fenton et al., *Adv Mater.* (2016) 28:2939; U.S. Pat. 10,201,618). An exemplary LNP formulation herein, Lipid A, contains OF-02.

- 15 In some embodiments, the cationic lipid is cKK-E10 (Dong et al., *PNAS* (2014) 111(11):3955-60; U.S. Pat. 9,512,073):



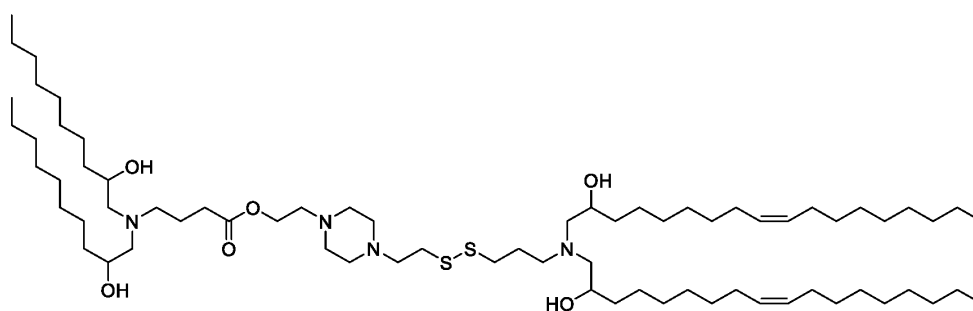
cKK-E10

Formula (II)

5 An exemplary LNP formulation herein, Lipid B, contains cKK-E10.

In some embodiments, the cationic lipid is GL-HEPES-E3-E10-DS-3-E18-1 (2-(4-(2-((3-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate), which is a HEPES-based disulfide cationic lipid with a piperazine core, having the Formula III:

10

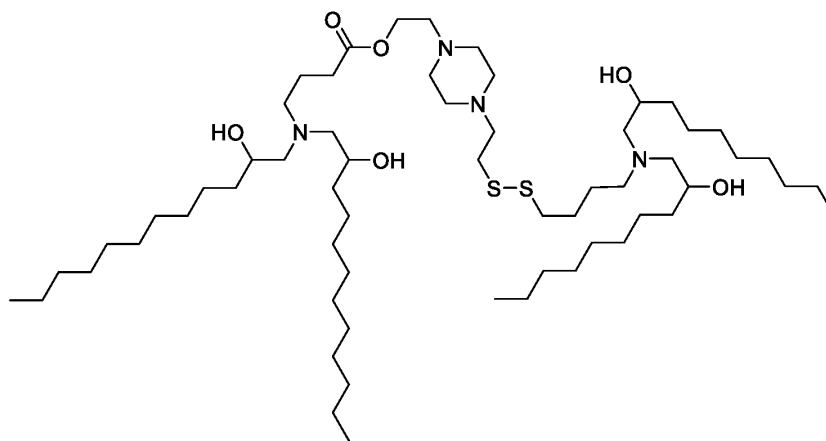


Formula (III)

An exemplary LNP formulation herein, Lipid C, contains GL-HEPES-E3-E10-DS-3-E18-1. Lipid C has the same composition as Lipid A or Lipid B but for the difference in the cationic lipid.

15 In some embodiments, the cationic lipid is GL-HEPES-E3-E12-DS-4-E10 (2-(4-(2-((3-(bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-

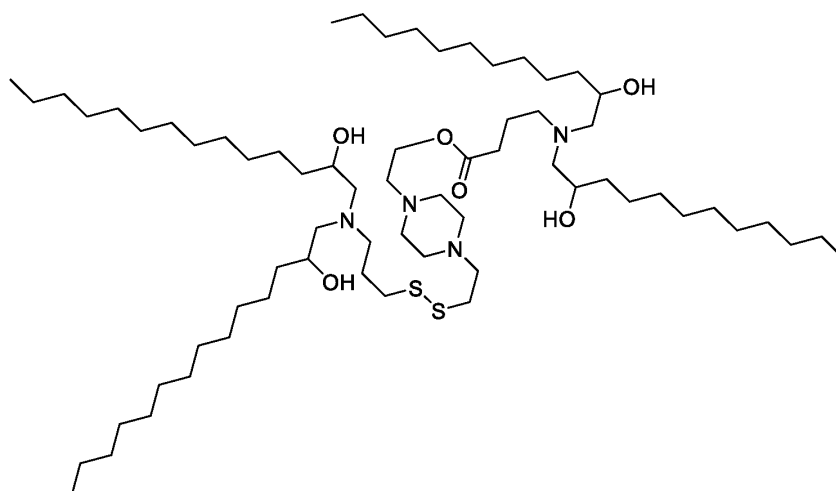
hydroxydodecyl)amino)butanoate), which is a HEPES-based disulfide cationic lipid with a piperazine core, having the Formula IV:



Formula (IV)

An exemplary LNP formulation herein, Lipid D, contains GL-HEPES-E3-E12-DS-4-E10. Lipid D has the same composition as Lipid A or Lipid B but for the difference in the cationic lipid.

In some embodiments, the cationic lipid is GL-HEPES-E3-E12-DS-3-E14 (2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate), which is a HEPES-based disulfide cationic lipid with a piperazine core, having the Formula V:



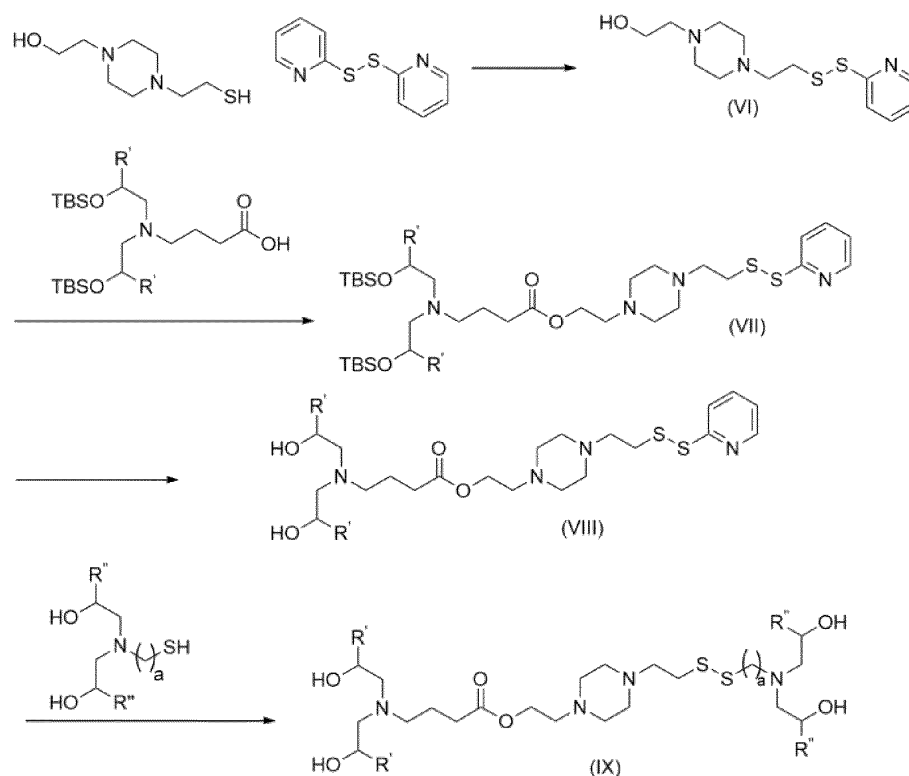
Formula (V)

An exemplary LNP formulation herein, Lipid E, contains GL-HEPES-E3-E12-DS-3-E14. Lipid E has the same composition as Lipid A or Lipid B but for the difference in the cationic lipid.

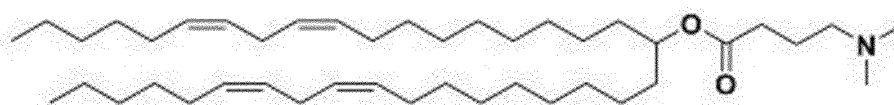
The cationic lipids GL-HEPES-E3-E10-DS-3-E18-1 (III), GL-HEPES-E3-E12-DS-4-E10 (IV), and GL-HEPES-E3-E12-DS-3-E14 (V) can be synthesized according to the general procedure set out in Scheme 1:

5

Scheme 1: General Synthetic Scheme for Lipids of Formulas (III), (IV), and (V)



In some embodiments, the cationic lipid is MC3, having the Formula VI:

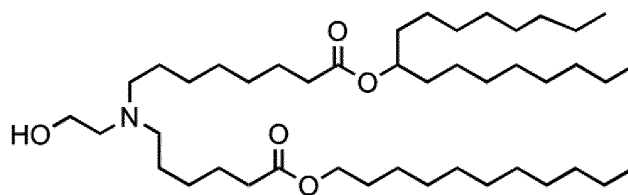


Formula (VI)

10

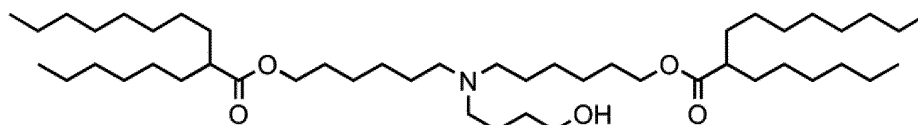
In some embodiments, the cationic lipid is SM-102 (9-heptadecanyl 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]octanoate), having the Formula VII:

60



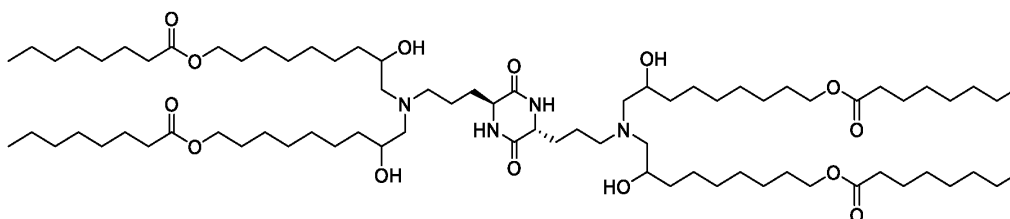
Formula (VII)

In some embodiments, the cationic lipid is ALC-0315 [(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl)bis(2-hexyldecanoate), having the Formula VIII:



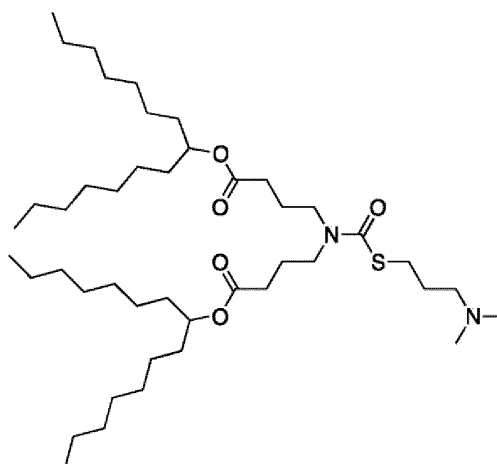
Formula (VIII)

In some embodiments, the cationic lipid is cOrn-EE1, having the Formula IX:



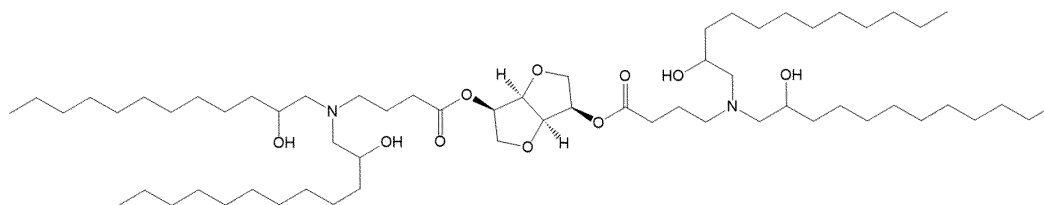
Formula (IX)

10 In some embodiments, the cationic lipid is ATX-126 (4,4'-[[[3-(dimethylamino)propyl]thio]carbonyl]imino]bis-butanoic acid, 1,1'-bis(1-heptyloctyl) ester), having the Formula X:



In some embodiments, the cationic lipid may be selected from the group comprising cKK-E10; OF-02; [(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl] 4-(dimethylamino)butanoate (D-Lin-MC3-DMA); 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA); 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane (DLin-DMA); di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319); 9-heptadecanoyl 8-((2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino)octanoate (SM-102); [(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate) (ALC-0315); [3-(dimethylamino)-2-[(Z)-octadec-9-enoyl]oxypropyl] (Z)-octadec-9-enoate (DODAP); 2,5-bis(3-aminopropylamino)-N-[2-[di(heptadecyl)amino]-2-oxoethyl]pentanamide (DOGS); [(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl] N-[2-(dimethylamino)ethyl]carbamate (DC-Chol); tetrakis(8-methylnonyl) 3,3',3'',3'''-(((methylazanediyl)bis(propane-3,1-diyl))bis(azanetriyl))tetrapropionate (306Oi10); decyl (2-(dioctylammonio)ethyl)phosphate (9A1P9); ethyl 5,5-di((Z)-heptadec-8-en-1-yl)-1-(3-(pyrrolidin-1-yl)propyl)-2,5-dihydro-1H-imidazole-2-carboxylate (A2-Iso5-2DC18); bis(2-(dodecylsulfanyl)ethyl) 3,3'-((3-methyl-9-oxo-10-oxa-13,14-dithia-3,6-diazahexacosyl)azanediyl)dipropionate (BAME-O16B); 1,1'-((2-(4-(2-((2-bis(2-hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl) piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200); 3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine-2,5-dione (cKK-E12); hexa(octan-3-yl) 9,9',9'',9''',9''''-((((benzene-1,3,5-tricarbonyl)tris(azanediy)) tris(propane-3,1-diyl)) tris(azanetriyl))hexanonanoate (FTT5); (((3,6-dioxopiperazine-2,5-diyl)bis(butane-4, 1-diyl))bis(azanetriyl))tetrakis(ethane-2,1-diyl) (9Z,9'Z,9''Z,9'''Z,12Z,12'Z,12''Z,12'''Z)-tetrakis (octadeca-9,12-dienoate) (OF-Deg-Lin); TT3; N¹,N³,N⁵-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide; N1-[2-((1S)-1-[(3-aminopropyl)amino]-4-[di(3-aminopropyl)amino]butylcarboxamido)ethyl]-3,4-di[oleyloxy]-benzamide (MVL5); heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Lipid 5); 4,4'-[[[3-(dimethylamino)propyl]thio]carbonyl]imino]bis-butanoic acid, 1,1'-bis(1-heptyloctyl) ester (ATX-126) GL-HEPES-E3-E10-DS-3-E18-1; GL-HEPES-E3-E12-DS-4-E10; GL-HEPES-E3-E12-DS-3-E14, IM-001; and combinations thereof.

In some embodiments, the cationic lipid is IM-001, having the Formula XI (EP23306049.0):



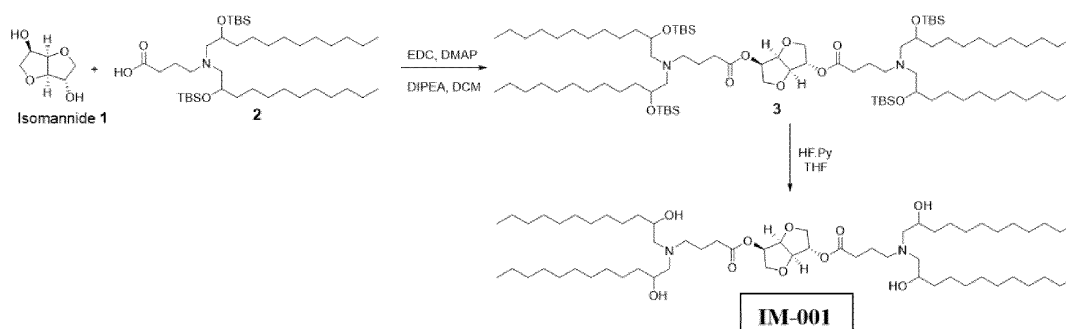
30

Formula (XI)

An exemplary LNP formulation herein, Lipid G, contains IM-001. Lipid G has the same composition as Lipid A or Lipid B but for the difference in the cationic lipid.

The cationic lipid IM-001 (XI) can be synthesized according to the general procedure set out in Scheme 2:

Scheme 2: General Synthetic Scheme for Lipid of Formula (XI)



5

Scheme 2 may be performed as described in Example 14.

In some embodiments, the cationic lipid is biodegradable.

In some embodiments, the cationic lipid is not biodegradable.

In some embodiments, the cationic lipid is cleavable.

10 In some embodiments, the cationic lipid is not cleavable.

Cationic lipids are described in further detail in Dong et al. (PNAS. 111(11):3955-60. 2014); Fenton et al. (Adv Mater. 28:2939. 2016); U.S. Pat. No. 9,512,073; and U.S. Pat. No. 10,201,618, each of which is incorporated herein by reference.

B. PEGylated Lipids

15 The PEGylated lipid component provides control over particle size and stability of the nanoparticle. The addition of such components may prevent complex aggregation and provide a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid pharmaceutical composition to target tissues (Klibanov et al. FEBS Letters 268(1):235-7. 1990). These components may be selected to rapidly exchange out of the pharmaceutical composition *in vivo* (see, e.g., U.S. Pat. No. 5,885,613).

20 Contemplated PEGylated lipids include, but are not limited to, a polyethylene glycol (PEG) chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C₆-C₂₀ (e.g., C₈, C₁₀, C₁₂, C₁₄, C₁₆, or C₁₈) length, such as a derivatized ceramide (e.g., N-octanoyl-sphingosine-1-[succinyl(methoxypolyethylene glycol)] (C8 PEG ceramide)). In some embodiments, the PEGylated lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (DMG-PEG); 1,2-distearoyl-sn-glycero-3-

phosphoethanolamine-polyethylene glycol (DSPE-PEG); 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine-polyethylene glycol (DLPE-PEG); or 1,2-distearoyl-rac-glycero-polyethelene glycol (DSG-PEG), PEG-DAG; PEG-PE; PEG-S-DAG; PEG-S-DMG; PEG-cer; a PEG-dialkoxypopylcarbamate; 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); and combinations thereof.

In certain embodiments, the PEG has a high molecular weight, e.g., 2000-2400 g/mol. In certain embodiments, the PEG is PEG2000 (or PEG-2K). In certain embodiments, the PEGylated lipid herein is DMG-PEG2000, DSPE-PEG2000, DLPE-PEG2000, DSG-PEG2000, C8 PEG2000, or ALC-0159 (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide). In certain embodiments, the PEGylated lipid herein is DMG-PEG2000.

C. Cholesterol-Based Lipids

The cholesterol component provides stability to the lipid bilayer structure within the nanoparticle. In some embodiments, the LNPs comprise one or more cholesterol-based lipids. Suitable cholesterol-based lipids include, for example: DC-Choi (N,N-dimethyl-N-ethylcarboxamidcholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao et al., *Biochem Biophys Res Comm.* (1991) 179:280; Wolf et al., *BioTechniques* (1997) 23:139; U.S. Pat. 5,744,335), imidazole cholesterol ester ("ICE"; WO2011/068810), sitosterol (22,23-dihydrostigmasterol), β -sitosterol, sitostanol, fucosterol, stigmasterol (stigmasta-5,22-dien-3-ol), ergosterol; desmosterol (3 β -hydroxy-5,24-cholestadiene); lanosterol (8,24-lanostadien-3 β -ol); 7-dehydrocholesterol (Δ 5,7-cholesterol); dihydrolanosterol (24,25-dihydrolanosterol); zymosterol (5 α -cholesta-8,24-dien-3 β -ol); lathosterol (5 α -cholest-7-en-3 β -ol); diosgenin ((3 β ,25R)-spirost-5-en-3-ol); campesterol (campest-5-en-3 β -ol); campestanol (5 α -campestan-3 β -ol); 24-methylene cholesterol (5,24(28)-cholestadien-24-methylen-3 β -ol); cholesteryl margarate (cholest-5-en-3 β -yl heptadecanoate); cholesteryl oleate; cholesteryl stearate and other modified forms of cholesterol. In some embodiments, the cholesterol-based lipid used in the LNPs is cholesterol.

D. Helper Lipids

A helper lipid enhances the structural stability of the LNP and helps the LNP in endosome escape. It improves uptake and release of the mRNA drug payload. In some embodiments, the helper lipid is a zwitterionic lipid, which has fusogenic properties for enhancing uptake and release of the drug payload. Examples of helper lipids are 1,2-dioleoyl-SN-glycero-3-phosphoethanolamine (DOPE); 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS); 1,2-dielaioyl-sn-glycero-3-phosphoethanolamine (DEPE); and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DPOC), dipalmitoylphosphatidylcholine (DPPC), DMPC, 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), 1,2-Distearoylphosphatidylethanolamine (DSPE), and 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE).

Other exemplary helper lipids are dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), phosphatidylserine, sphingolipids, sphingomyelins, ceramides, cerebroside, gangliosides, 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), or a combination thereof. In certain embodiments, the helper lipid is DOPE. In certain embodiments, the helper lipid is DSPC.

In various embodiments, the present LNPs comprise (i) a cationic lipid selected from OF-02, cKK-E10, GL-HEPES-E3-E10-DS-3-E18-1, GL-HEPES-E3-E12-DS-4-E10, GL-HEPES-E3-E12-DS-3-E14, or IM-001; (ii) DMG-PEG2000; (iii) cholesterol; and (iv) DOPE.

In other embodiments, the present LNPs comprise (i) SM-102, (ii) DMG-PEG2000, (iii) cholesterol, and (iv) DSPC.

In yet other embodiments, the present LNPs comprise (i) ALC-0315, (ii) ALC-0159, (iii) cholesterol, and (iv) DSPC.

In yet other embodiments, the present LNPs comprise (i) ATX-126, (ii) DMG-PEG2000, (iii) cholesterol, and (iv) DSPC.

E. Molar Ratios of the Lipid Components

The molar ratios of the above components are important for the LNPs' effectiveness in delivering mRNA. The molar ratio of the cationic lipid, the PEGylated lipid, the cholesterol-based lipid, and the helper lipid is A: B: C: D, where $A + B + C + D = 100\%$. In some embodiments, the molar ratio of the cationic lipid in the LNPs relative to the total lipids (i.e., A) is 35-55%, such as 35-50% (e.g., 38-42% such as 40%, or 45-50%). In some embodiments, the molar ratio of the PEGylated lipid component relative to the total lipids (i.e., B) is 0.25-2.75% (e.g., 1-2% such as 1.5%). In some embodiments, the molar ratio of the cholesterol-based lipid relative to the total lipids (i.e., C) is 20-50% (e.g., 27-30% such as 28.5%, or 38-43%). In some embodiments, the molar ratio of the helper lipid relative to the total lipids (i.e., D) is 5-35% (e.g., 28-32% such as 30%, or 8-12%, such as 10%). In some embodiments, the (PEGylated lipid + cholesterol) components have the same molar amount as the helper lipid. In some embodiments, the LNPs contain a molar ratio of the cationic lipid to the helper lipid that is more than 1.

In certain embodiments, the LNP of the disclosure comprises:

a cationic lipid at a molar ratio of 35% to 55% or 40% to 50% (e.g., a cationic lipid at a molar ratio of 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, or 55%);

a polyethylene glycol (PEG) conjugated (PEGylated) lipid at a molar ratio of 0.25% to 2.75% or 1.00% to 2.00% (e.g., a PEGylated lipid at a molar ratio of 0.25%, 0.50%, 0.75%, 1.00%, 1.25%, 1.50%, 1.75%, 2.00%, 2.25%, 2.50%, or 2.75%);

5 a cholesterol-based lipid at a molar ratio of 20% to 50%, 25% to 45%, or 28.5% to 43% (e.g., a cholesterol-based lipid at a molar ratio of 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50%); and

10 a helper lipid at a molar ratio of 5% to 35%, 8% to 30%, or 10% to 30% (e.g., a helper lipid at a molar ratio of 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, or 35%),

wherein all of the molar ratios are relative to the total lipid content of the LNP.

In certain embodiments, the LNP comprises: a cationic lipid at a molar ratio of 40%; a PEGylated lipid at a molar ratio of 1.5%; a cholesterol-based lipid at a molar ratio of 28.5%; and a helper lipid at a molar ratio of 30%.

15 In certain embodiments, the LNP of the disclosure comprises: a cationic lipid at a molar ratio of 45 to 50%; a PEGylated lipid at a molar ratio of 1.5 to 1.7%; a cholesterol-based lipid at a molar ratio of 38 to 43%; and a helper lipid at a molar ratio of 9 to 10%.

In certain embodiments, the PEGylated lipid is dimyristoyl-PEG2000 (DMG-PEG2000).

In various embodiments, the cholesterol-based lipid is cholesterol.

20 In some embodiments, the helper lipid is 1,2-dioleoyl-SN-glycero-3-phosphoethanolamine (DOPE).

In certain embodiments, the LNP comprises: OF-02 at a molar ratio of 35% to 55%; DMG-PEG2000 at a molar ratio of 0.25% to 2.75%; cholesterol at a molar ratio of 20% to 50%; and DOPE at a molar ratio of 5% to 35%.

25 In certain embodiments, the LNP comprises: cKK-E10 at a molar ratio of 35% to 55%; DMG-PEG2000 at a molar ratio of 0.25% to 2.75%; cholesterol at a molar ratio of 20% to 50%; and DOPE at a molar ratio of 5% to 35%.

In certain embodiments, the LNP comprises: GL-HEPES-E3-E10-DS-3-E18-1 at a molar ratio of 35% to 55%; DMG-PEG2000 at a molar ratio of 0.25% to 2.75%; cholesterol at a molar ratio of 20% to 50%; and DOPE at a molar ratio of 5% to 35%.

In certain embodiments, the LNP comprises: GL-HEPES-E3-E12-DS-4-E10 at a molar ratio of 35% to 55%; DMG-PEG2000 at a molar ratio of 0.25% to 2.75%; cholesterol at a molar ratio of 20% to 50%; and DOPE at a molar ratio of 5% to 35%.

5 In certain embodiments, the LNP comprises: GL-HEPES-E3-E12-DS-3-E14 at a molar ratio of 35% to 55%; DMG-PEG2000 at a molar ratio of 0.25% to 2.75%; cholesterol at a molar ratio of 20% to 50%; and DOPE at a molar ratio of 5% to 35%.

In certain embodiments, the LNP comprises: SM-102 at a molar ratio of 35% to 55%; DMG-PEG2000 at a molar ratio of 0.25% to 2.75%; cholesterol at a molar ratio of 20% to 50%; and DSPC at a molar ratio of 5% to 35%.

10 In certain embodiments, the LNP comprises: ALC-0315 at a molar ratio of 35% to 55%; ALC-0159 at a molar ratio of 0.25% to 2.75%; cholesterol at a molar ratio of 20% to 50%; and DSPC at a molar ratio of 5% to 35%.

15 In certain embodiments, the LNP comprises: OF-02 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%. This LNP formulation is designated "Lipid A" herein.

In certain embodiments, the LNP comprises: cKK-E10 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%. This LNP formulation is designated "Lipid B" herein.

20 In certain embodiments, the LNP comprises: GL-HEPES-E3-E10-DS-3-E18-1 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%. This LNP formulation is designated "Lipid C" herein.

In certain embodiments, the LNP comprises: GL-HEPES-E3-E12-DS-4-E10 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%. This LNP formulation is designated "Lipid D" herein.

25 In certain embodiments, the LNP comprises: GL-HEPES-E3-E12-DS-3-E14 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%. This LNP formulation is designated "Lipid E" herein.

30 In certain embodiments, the LNP comprises DLin-MC3-DMA (MC3) at a molar ratio of 50%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 38.5%; and DSPC at a molar ratio of 10%. This LNP formulation is designated "Lipid F" herein.

In certain embodiments, the LNP comprises: IM-001 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%. This LNP formulation is designated “Lipid G” herein.

5 In certain embodiments, the LNP comprises: 9-heptadecanyl 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]octanoate (SM-102) at a molar ratio of 50%; 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) at a molar ratio of 10%; cholesterol at a molar ratio of 38.5%; and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000) at a molar ratio of 1.5%.

10 In certain embodiments, the LNP comprises: (4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate) (ALC-0315) at a molar ratio of 46.3%; 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) at a molar ratio of 9.4%; cholesterol at a molar ratio of 42.7%; and 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a molar ratio of 1.6%.

15 In certain embodiments, the LNP comprises: (4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate) (ALC-0315) at a molar ratio of 47.4%; 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) at a molar ratio of 10%; cholesterol at a molar ratio of 40.9%; and 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a molar ratio of 1.7%.

In certain embodiments, the LNP comprises 4,4'-[[[3-(dimethylamino)propyl]thio]carbonyl]imino]bis-butanoic acid, 1,1'-bis(1-heptyloctyl) ester (ATX-126) at a molar ratio of 50%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 38.5%; and DSPC at a molar ratio of 10%.

20 In some embodiments, the LNP formulation is as defined for “Lipid A”, “Lipid B”, “Lipid D”, or Lipid G (e.g., Lipid D or Lipid G).

To calculate the actual amount of each lipid to be put into an LNP formulation, the molar amount of the cationic lipid is first determined based on a desired N/P ratio, where N is the number of nitrogen atoms in the cationic lipid and P is the number of phosphate groups in the mRNA to be transported by the LNP. Next, the molar amount of each of the other lipids is calculated based on the molar amount of the cationic lipid and the molar ratio selected. These molar amounts are then converted to weights using the molecular weight of each lipid.

25

F. Nucleic acids within LNPs

The LNP compositions described herein may comprise a nucleic acid (e.g., a mRNA) of the present invention.

30 Where desired, the LNP may be multi-valent. In some embodiments, the LNP may carry nucleic acids, such as mRNAs, that encode more than one polypeptide of the present invention, such as two, three, four, five, six, seven, or eight polypeptides. For example, the LNP may carry multiple nucleic acids of the present

invention (e.g., mRNA), each encoding a different polypeptide of the invention; or carry a polycistronic mRNA that can be translated into more than one polypeptide of the invention (e.g., each antigen-coding sequence is separated by a nucleotide linker encoding a self-cleaving peptide such as a 2A peptide). An LNP carrying different nucleic acids (e.g., mRNA) typically comprises (encapsulate) multiple copies of each nucleic acid. For example, an LNP carrying or encapsulating two different nucleic acids typically carries multiple copies of each of the two different nucleic acids.

In some embodiments, two or more (e.g., two, three or four) nucleic acids (e.g., mRNAs) as described herein encoding different polypeptides as described herein are co-encapsulated in the same LNP. For example, the LNPs described herein may co-encapsulate (a) a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein, (b) a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein, and (c) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein. In some embodiments, the LNPs described herein co-encapsulate (a) a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein, (b) a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein, and (d) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein. In some embodiments, the LNPs described herein co-encapsulate (a) a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein, (b) a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein, (c) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein, and (d) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein.

Any two nucleic acids (e.g., two mRNAs) as described herein may be present in a weight ratio of 1:1. Any three nucleic acids (e.g., three mRNAs) as described herein may be present in a weight ratio of 1:1:1. Any four nucleic acids (e.g., four mRNAs) as described herein may be present in a weight ratio of 1:1:1:1.

In some embodiments, the LNP is as described herein (e.g., Lipid D or Lipid G).

Alternatively, any two or more (e.g., four) nucleic acids (e.g., mRNAs) encoding different polypeptides as described herein are encapsulated in separate LNPs.

In some embodiments, a single LNP formulation may comprise multiple kinds (e.g., two, three, four, five, six, seven, eight, nine, ten, or more) of LNPs, each kind carrying a different nucleic acid (e.g., mRNA).

When the nucleic acid is mRNA, the mRNA may be unmodified (i.e., containing only natural ribonucleotides A, U, C, and/or G linked by phosphodiester bonds), or chemically modified (e.g., including nucleotide analogs such as pseudouridines (e.g., N-1-methyl pseudouridine), 2'-fluoro ribonucleotides, and

2'-methoxy ribonucleotides, and/or phosphorothioate bonds). The mRNA molecule may comprise a 5' cap and a polyA tail.

G. Buffer and Other Components

To stabilize the nucleic acid and/or LNPs (e.g., to prolong the shelf-life of the vaccine product), to facilitate administration of the LNP pharmaceutical composition, and/or to enhance in vivo expression of the nucleic acid, the nucleic acid and/or LNP can be formulated in combination with one or more carriers, targeting ligands, stabilizing reagents (e.g., preservatives and antioxidants), and/or other pharmaceutically acceptable excipients. Examples of such excipients are parabens, thimerosal, thiomersal, chlorobutanol, benzalkonium chloride, and chelators (e.g., EDTA).

The LNP compositions of the present disclosure can be provided as a frozen liquid form or a lyophilized form. A variety of cryoprotectants may be used, including, without limitations, sucrose, trehalose, glucose, mannitol, mannose, dextrose, and the like. The cryoprotectant may constitute 5-30% (w/v) of the LNP composition. In some embodiments, the LNP composition comprises trehalose, e.g., at 5-30% (e.g., 10%) (w/v). Once formulated with the cryoprotectant, the LNP compositions may be frozen (or lyophilized and cryopreserved) at -20°C to -80°C.

The LNP compositions may be provided to a patient in an aqueous buffered solution – thawed if previously frozen, or if previously lyophilized, reconstituted in an aqueous buffered solution at bedside. The buffered solution preferably is isotonic and suitable for e.g., intramuscular or intradermal injection. In some embodiments, the buffered solution is a phosphate-buffered saline (PBS).

Nucleic acids

A nucleic acid of the invention may be RNA or DNA. The nucleic acids of the invention may be single or double-stranded. In certain embodiments, the nucleic acid is RNA, e.g., mRNA.

mRNA

In some embodiments, the nucleic acids of the present invention are messenger RNAs (mRNAs). mRNAs can be modified or unmodified. mRNAs may contain one or more coding and non-coding regions. A coding region is alternatively referred to as an open reading frame (ORF). Non-coding regions in an mRNA include the 5' cap, 5' untranslated region (UTR), 3' UTR, and a polyA tail. An mRNA can be purified from natural sources, produced using recombinant expression systems (e.g., *in vitro* transcription) and optionally purified, or chemically synthesised.

In certain embodiments, the mRNA comprises an ORF encoding an antigen of interest. In certain embodiments, the RNA (e.g., mRNA) further comprises at least one 5' UTR, 3' UTR, a poly(A) tail, and/or a 5' cap. In some embodiments, the mRNA comprises (i) a 5' cap as defined herein; (ii) a 5' untranslated region (UTR) as defined herein; (iii) a protein coding region; (iv) a 3' UTR as defined herein; and (v) a

polyA tail. Typically, the 3' end of (i) bonds directly to the 5' end of (ii) via a 3' to 5' phosphodiester linkage; the 3' end of (ii) bonds directly to the 5' end of (iii) via a 3' to 5' phosphodiester linkage; the 3' end of (iii) bonds directly to the 5' end of (iv) via a 3' to 5' phosphodiester linkage; and the 3' end of (iv) bonds directly to the 5' end of (v) via a 3' to 5' phosphodiester linkage.

- 5 In certain embodiments, the mRNA comprises at least one, at least two, at least three or more stop codon(s), wherein the stop codon(s) can be selected from UAA, UGA and UAG, and wherein the at least two, at least three or more stop codon(s) can be identical or different. Typically, the at least one stop codon comprises UAA or UGA (e.g., UAA). Typically, the at least two stop codons comprise at least two identical stop codons, such as UAA or UGA (e.g., UAAUAA or UGAUGA) or at least two different stop codons, which
- 10 may in particular be selected from UAA and UGA (e.g., UGAUAA). Typically, the at least three stop codons comprise UAA, UGA and UAG (e.g., UGAUAAUAG).

An mRNA sequence is presented in the 5' to 3' direction unless otherwise indicated.

5' Cap

- 15 An mRNA 5' cap can provide resistance to nucleases found in most eukaryotic cells and promote translation efficiency. Several types of 5' caps are known. A 7-methylguanosine cap (also referred to as "m⁷G" or "Cap-0"), comprises a guanosine that is linked through a 5' – 5' - triphosphate bond to the first transcribed nucleotide.

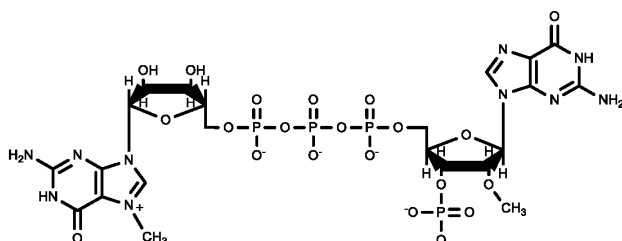
- A 5' cap is typically added as follows: first, an RNA terminal phosphatase removes one of the terminal phosphate groups from the 5' nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP)
- 20 is then added to the terminal phosphates via a guanylyl transferase, producing a 5' – 5' triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. Examples of cap structures include, but are not limited to, m⁷G(5')ppp, (5'(A,G(5'))ppp(5')A, and G(5')ppp(5')G. Additional cap structures are described in U.S. Publication No. US 2016/0032356 and U.S. Publication No. US 2018/0125989, which are incorporated herein by reference.

- 25 5'-capping of polynucleotides may be completed concomitantly during the *in vitro*-transcription reaction using the following chemical RNA cap analogs to generate the 5'-guanosine cap structure according to manufacturer protocols: 3'-O-Me-m⁷G(5')ppp(5')G (the ARCA cap); G(5')ppp(5')A; G(5')ppp(5')G; m⁷G(5')ppp(5')A; m⁷G(5')ppp(5')G; m⁷G(5')ppp(5')(2'OMeA)pG; m⁷G(5')ppp(5')(2'OMeA)pU; m⁷G(5')ppp(5')(2'OMeG)pG (New England BioLabs, Ipswich, MA; TriLink Biotechnologies). 5'-capping
- 30 of modified RNA may be completed post-transcriptionally using a vaccinia virus capping enzyme to generate the Cap 0 structure: m⁷G(5')ppp(5')G. Cap 1 structure may be generated using both vaccinia virus capping enzyme and a 2'-O methyl-transferase to generate: m⁷G(5')ppp(5')G-2'-O-methyl. Cap 2 structure may be generated from the Cap 1 structure followed by the 2'-O-methylation of the 5'-antepenultimate nucleotide using a 2'-O methyl-transferase. Cap 3 structure may be generated from the

Cap 2 structure followed by the 2'-O-methylation of the 5'-preantepenultimate nucleotide using a 2'-O methyl-transferase.

In certain embodiments, the mRNA of the invention comprises a 5' cap selected from the group consisting of 3'-O-Me-m7G(5')ppp(5')G (the ARCA cap), G(5')ppp(5')A, G(5')ppp(5')G, m7G(5')ppp(5')A, m7G(5')ppp(5')G, m7G(5')ppp(5')(2'OMeA)pG, m7G(5')ppp(5')(2'OMeA)pU, and m7G(5')ppp(5')(2'OMeG)pG.

In certain embodiments, the mRNA of the invention comprises a 5' cap of:



10 *Untranslated Region (UTR)*

In some embodiments, the mRNA of the invention includes a 5' and/or 3' untranslated region (UTR). In mRNA, the 5' UTR starts at the transcription start site and continues to the start codon but does not include the start codon. The 3' UTR starts immediately following the stop codon and continues until the transcriptional termination signal.

15 In some embodiments, the mRNA disclosed herein may comprise a 5' UTR that includes one or more elements that affect an mRNA's stability or translation. In some embodiments, a 5' UTR may be about 10 to 5,000 nucleotides in length. In some embodiments, a 5' UTR may be about 50 to 500 nucleotides in length. In some embodiments, the 5' UTR is at least about 10 nucleotides in length, about 20 nucleotides in length, about 30 nucleotides in length, about 40 nucleotides in length, about 50 nucleotides in length, about 100 nucleotides in length, about 150 nucleotides in length, about 200 nucleotides in length, about 250 nucleotides in length, about 300 nucleotides in length, about 350 nucleotides in length, about 400 nucleotides in length, about 450 nucleotides in length, about 500 nucleotides in length, about 550 nucleotides in length, about 600 nucleotides in length, about 650 nucleotides in length, about 700 nucleotides in length, about 750 nucleotides in length, about 800 nucleotides in length, about 850 nucleotides in length, about 900 nucleotides in length, about 950 nucleotides in length, about 1,000 nucleotides in length, about 1,500 nucleotides in length, about 2,000 nucleotides in length, about 2,500 nucleotides in length, about 3,000 nucleotides in length, about 3,500 nucleotides in length, about 4,000 nucleotides in length, about 4,500 nucleotides in length or about 5,000 nucleotides in length.

In some embodiments, the mRNA disclosed herein may comprise a 3' UTR comprising one or more of a polyadenylation signal, a binding site for proteins that affect an mRNA's stability of location in a cell, or one or more binding sites for miRNAs. In some embodiments, a 3' UTR may be 50 to 5,000 nucleotides in length or longer. In some embodiments, a 3' UTR may be 50 to 1,000 nucleotides in length or longer. In some embodiments, the 3' UTR is at least about 50 nucleotides in length, about 100 nucleotides in length, about 150 nucleotides in length, about 200 nucleotides in length, about 250 nucleotides in length, about 300 nucleotides in length, about 350 nucleotides in length, about 400 nucleotides in length, about 450 nucleotides in length, about 500 nucleotides in length, about 550 nucleotides in length, about 600 nucleotides in length, about 650 nucleotides in length, about 700 nucleotides in length, about 750 nucleotides in length, about 800 nucleotides in length, about 850 nucleotides in length, about 900 nucleotides in length, about 950 nucleotides in length, about 1,000 nucleotides in length, about 1,500 nucleotides in length, about 2,000 nucleotides in length, about 2,500 nucleotides in length, about 3,000 nucleotides in length, about 3,500 nucleotides in length, about 4,000 nucleotides in length, about 4,500 nucleotides in length, or about 5,000 nucleotides in length.

In some embodiments, the mRNA disclosed herein may comprise a 5' or 3' UTR that is derived from a gene distinct from the one encoded by the mRNA transcript (i.e., the UTR is a heterologous UTR).

In certain embodiments, the 5' and/or 3' UTR sequences can be derived from mRNA which are stable (e.g., globin, actin, GAPDH, tubulin, histone, or citric acid cycle enzymes) to increase the stability of the mRNA. For example, a 5' UTR sequence may include a partial sequence of a CMV immediate-early 1 (IE1) gene, or a fragment thereof, to improve the nuclease resistance and/or improve the half-life of the mRNA. Also contemplated is the inclusion of a sequence encoding human growth hormone (hGH), or a fragment thereof, to the 3' end or untranslated region of the mRNA. Generally, these modifications improve the stability and/or pharmacokinetic properties (e.g., half-life) of the mRNA relative to their unmodified counterparts, and include, for example, modifications made to improve such mRNA resistance to *in vivo* nuclease digestion.

Exemplary 5' UTRs include a sequence derived from a CMV immediate-early 1 (IE1) gene (U.S. Publication Nos. 2014/0206753 and 2015/0157565, each of which is incorporated herein by reference), or the sequence GGGAUCCUACC (SEQ ID NO: 837) (U.S. Publication No. 2016/0151409, incorporated herein by reference).

In various embodiments, the 5' UTR may be derived from the 5' UTR of a TOP gene. TOP genes are typically characterized by the presence of a 5'-terminal oligopyrimidine (TOP) tract. Furthermore, most TOP genes are characterized by growth-associated translational regulation. However, TOP genes with a tissue specific translational regulation are also known. In certain embodiments, the 5' UTR derived from the 5' UTR of a TOP gene lacks the 5' TOP motif (the oligopyrimidine tract) (e.g., U.S. Publication Nos.

2017/0029847, 2016/0304883, 2016/0235864, and 2016/0166710, each of which is incorporated herein by reference).

In certain embodiments, the 5' UTR is derived from a ribosomal protein Large 32 (L32) gene (U.S. Publication No. 2017/0029847, *supra*).

- 5 In certain embodiments, the 5' UTR is derived from the 5' UTR of an hydroxysteroid (17-b) dehydrogenase
4 gene (HSD17B4) (U.S. Publication No. 2016/0166710, *supra*).

In certain embodiments, the 5' UTR is derived from the 5' UTR of an ATP5A1 gene (U.S. Publication No. 2016/0166710, *supra*).

In some embodiments, an internal ribosome entry site (IRES) is used instead of a 5' UTR.

- 10 In some embodiments, the 5'UTR comprises a nucleic acid sequence set forth in SEQ ID NO: 838 and reproduced below:

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUGCAUAGAAGACACCGGGAC
CGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUCCCCGUGCCAAGAGU
GACUCACCGUCCUUGACACG (SEO ID NO: 838).

- 15 In some embodiments, the 3'UTR comprises a nucleic acid sequence set forth in SEQ ID NO: 839 and reproduced below:

CGGGUGGCAUCCCUUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCUGGAAGUUGCCACUCC
AGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUC (SEQ ID NO: 839).

- The 5' UTR and 3'UTR are described in further detail in WO2012/075040, incorporated herein by
20 reference.

Polyadenylated Tail

As used herein, the terms “poly(A) sequence,” “poly(A) tail,” and “poly(A) region” refer to a sequence of adenosine nucleotides at the 3’ end of the mRNA molecule. The poly(A) tail may confer stability to the mRNA and protect it from exonuclease degradation. The poly(A) tail may enhance translation. In some
25 embodiments, the poly(A) tail is essentially homopolymeric. For example, a poly(A) tail of 100 adenosine nucleotides may have essentially a length of 100 nucleotides. In certain embodiments, the poly(A) tail may be interrupted by at least one nucleotide different from an adenosine nucleotide (e.g., a nucleotide that is not an adenosine nucleotide). For example, a poly(A) tail of 100 adenosine nucleotides may have a length of more than 100 nucleotides (comprising 100 adenosine nucleotides and at least one nucleotide, or a stretch
30 of nucleotides, that are different from an adenosine nucleotide). In certain embodiments, the poly(A) tail comprises the sequence

AAAAAAAAAAAAAAAAAAAAAAAAAGCAUAUGACUAAAAAAAAAAAAAAAAAAAAA

AA (SEQ ID NO:840).

The “poly(A) tail,” as used herein, typically relates to RNA. However, in the context of the disclosure, the term likewise relates to corresponding sequences in a DNA molecule (e.g., a “poly(T) sequence”).

5 The poly(A) tail may comprise about 10 to about 500 adenosine nucleotides, about 10 to about 200 adenosine nucleotides, about 40 to about 200 adenosine nucleotides, or about 40 to about 150 adenosine nucleotides. The length of the poly(A) tail may be at least about 10, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, or 500 adenosine nucleotides. In some embodiments, the polyA tail comprises at least 50, at least 75 or at least 100 adenosine nucleotides.

10 In some embodiments where the nucleic acid is an RNA, the poly(A) tail of the nucleic acid is obtained from a DNA template during RNA *in vitro* transcription. In certain embodiments, the poly(A) tail is obtained *in vitro* by common methods of chemical synthesis without being transcribed from a DNA template. In various embodiments, poly(A) tails are generated by enzymatic polyadenylation of the RNA (after RNA *in vitro* transcription) using commercially available polyadenylation kits and corresponding
15 protocols, or alternatively, by using immobilized poly(A) polymerases, e.g., using methods and means as described in WO2016/174271.

The nucleic acid may comprise a poly(A) tail obtained by enzymatic polyadenylation, wherein the majority of nucleic acid molecules comprise about 100 (+/-20) to about 500 (+/-50) or about 250 (+/-20) adenosine nucleotides.

20 In some embodiments, the nucleic acid may comprise a poly(A) tail derived from a template DNA and may additionally comprise at least one additional poly(A) tail generated by enzymatic polyadenylation, e.g., as described in WO2016/091391.

In certain embodiments, the nucleic acid comprises at least one polyadenylation signal.

In various embodiments, the nucleic acid may comprise at least one poly(C) sequence.

25 The term “poly(C) sequence,” as used herein, is intended to be a sequence of cytosine nucleotides of up to about 200 cytosine nucleotides. In some embodiments, the poly(C) sequence comprises about 10 to about 200 cytosine nucleotides, about 10 to about 100 cytosine nucleotides, about 20 to about 70 cytosine nucleotides, about 20 to about 60 cytosine nucleotides, or about 10 to about 40 cytosine nucleotides. In some embodiments, the poly(C) sequence comprises about 30 cytosine nucleotides.

30 *Chemical Modification*

The mRNA disclosed herein may be modified or unmodified. Typically, the mRNA comprises at least one chemical modification. In some embodiments, the mRNA disclosed herein may contain one or more

modifications that typically enhance RNA stability. Exemplary modifications can include backbone modifications, sugar modifications, or base modifications. In some embodiments, the disclosed mRNA may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A) and guanine (G)) or pyrimidines (thymine (T), cytosine (C), and uracil (U)). In certain embodiments, the disclosed mRNA may be synthesized from modified nucleotide analogues or derivatives of purines and pyrimidines, such as, e.g., 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxy acetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queosine, β -D-mannosyl-queosine, phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine, and inosine.

In some embodiments, the disclosed mRNA may comprise at least one chemical modification including, but not limited to, pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-azauridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-azauridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, and 2'-O-methyluridine.

In some embodiments, the chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5-methoxyuridine, and a combination thereof.

In some embodiments, the chemical modification comprises N1-methylpseudouridine. Typically, the chemical modification comprises N1-methylpseudouridine. Typically, the chemical modification comprises N1-methylpseudouridine in place of every uridine, i.e. 100% of U residues are N1-methylpseudouridine.

In some embodiments, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% of the uracil nucleotides in the mRNA are chemically modified.

In some embodiments, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% of the uracil nucleotides in the ORF are chemically modified.

The preparation of such analogues is described, e.g., in U.S. Pat. No. 4,373,071, U.S. Pat. No. 4,401,796, U.S. Pat. No. 4,415,732, U.S. Pat. No. 4,458,066, U.S. Pat. No. 4,500,707, U.S. Pat. No. 4,668,777, U.S. Pat. No. 4,973,679, U.S. Pat. No. 5,047,524, U.S. Pat. No. 5,132,418, U.S. Pat. No. 5,153,319, U.S. Pat. No. 5,262,530, and U.S. Pat. No. 5,700,642.

mRNA Synthesis

The mRNAs disclosed herein may be synthesized according to any of a variety of methods. For example, mRNAs according to the present disclosure may be synthesized via *in vitro* transcription (IVT). Some methods for *in vitro* transcription are described, e.g., in Geall et al. (2013) Semin. Immunol. 25(2): 152-159; Brunelle et al. (2013) Methods Enzymol. 530:101-14. Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, an appropriate RNA polymerase (e.g., T3, T7, or SP6 RNA polymerase), DNase I, pyrophosphatase, and/or RNase inhibitor. The exact conditions may vary according to the specific application. The presence of these reagents is generally undesirable in a final mRNA product and these reagents can be considered impurities or contaminants which can be purified or removed to provide a clean and/or homogeneous mRNA that is suitable for therapeutic use. While mRNA provided from *in vitro* transcription reactions may be desirable in some embodiments, other sources of mRNA can be used according to the instant disclosure including wild-type mRNA produced from bacteria, fungi, plants, and/or animals.

Processes for Making Present LNP Compositions

The present LNPs can be prepared by various techniques presently known in the art. For example, multilamellar vesicles (MLV) may be prepared according to conventional techniques, such as by depositing a selected lipid on the inside wall of a suitable container or vessel by dissolving the lipid in an appropriate solvent, and then evaporating the solvent to leave a thin film on the inside of the vessel or by spray drying. An aqueous phase may then be added to the vessel with a vortexing motion that results in the formation of MLVs. Unilamellar vesicles (ULV) can then be formed by homogenization, sonication or extrusion of the multilamellar vesicles. In addition, unilamellar vesicles can be formed by detergent removal techniques.

Various methods are described in US 2011/0244026, US 2016/0038432, US 2018/0153822, US 2018/0125989, and PCT/US2020/043223 (filed July 23, 2020) and can be used to practice the present disclosure. One exemplary process entails encapsulating mRNA by mixing it with a mixture of lipids, without first pre-forming the lipids into lipid nanoparticles, as described in US 2016/0038432. Another

exemplary process entails encapsulating mRNA by mixing pre-formed LNPs with mRNA, as described in US 2018/0153822.

In some embodiments, the process of preparing mRNA-loaded LNPs includes a step of heating one or more of the solutions to a temperature greater than ambient temperature, the one or more solutions being the solution comprising the pre-formed lipid nanoparticles, the solution comprising the mRNA and the mixed solution comprising the LNP-encapsulated mRNA. In some embodiments, the process includes the step of heating one or both of the mRNA solution and the pre-formed LNP solution, prior to the mixing step. In some embodiments, the process includes heating one or more of the solutions comprising the pre-formed LNPs, the solution comprising the mRNA and the solution comprising the LNP-encapsulated mRNA, during the mixing step. In some embodiments, the process includes the step of heating the LNP-encapsulated mRNA, after the mixing step. In some embodiments, the temperature to which one or more of the solutions is heated is or is greater than about 30°C, 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, or 70°C. In some embodiments, the temperature to which one or more of the solutions is heated ranges from about 25-70°C, about 30-70°C, about 35-70°C, about 40-70°C, about 45-70°C, about 50-70°C, or about 60-70°C. In some embodiments, the temperature is about 65°C.

Various methods may be used to prepare an mRNA solution suitable for the present disclosure. In some embodiments, mRNA may be directly dissolved in a buffer solution described herein. In some embodiments, an mRNA solution may be generated by mixing an mRNA stock solution with a buffer solution prior to mixing with a lipid solution for encapsulation. In some embodiments, an mRNA solution may be generated by mixing an mRNA stock solution with a buffer solution immediately before mixing with a lipid solution for encapsulation. In some embodiments, a suitable mRNA stock solution may contain mRNA in water or a buffer at a concentration at or greater than about 0.2 mg/ml, 0.4 mg/ml, 0.5 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1.0 mg/ml, 1.2 mg/ml, 1.4 mg/ml, 1.5 mg/ml, or 1.6 mg/ml, 2.0 mg/ml, 2.5 mg/ml, 3.0 mg/ml, 3.5 mg/ml, 4.0 mg/ml, 4.5 mg/ml, or 5.0 mg/ml.

In some embodiments, an mRNA stock solution is mixed with a buffer solution using a pump. Exemplary pumps include but are not limited to gear pumps, peristaltic pumps and centrifugal pumps. Typically, the buffer solution is mixed at a rate greater than that of the mRNA stock solution. For example, the buffer solution may be mixed at a rate at least 1x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 15x, or 20x greater than the rate of the mRNA stock solution. In some embodiments, a buffer solution is mixed at a flow rate ranging between about 100-6000 ml/minute (e.g., about 100-300 ml/minute, 300-600 ml/minute, 600-1200 ml/minute, 1200-2400 ml/minute, 2400-3600 ml/minute, 3600-4800 ml/minute, 4800-6000 ml/minute, or 60-420 ml/minute). In some embodiments, a buffer solution is mixed at a flow rate of, or greater than, about 60 ml/minute, 100 ml/minute, 140 ml/minute, 180 ml/minute, 220 ml/minute, 260 ml/minute, 300 ml/minute, 340 ml/minute, 380 ml/minute, 420 ml/minute, 480 ml/minute, 540 ml/minute, 600 ml/minute, 1200 ml/minute, 2400 ml/minute, 3600 ml/minute, 4800 ml/minute, or 6000 ml/minute.

In some embodiments, an mRNA stock solution is mixed at a flow rate ranging between about 10-600 ml/minute (e.g., about 5-50 ml/minute, about 10-30 ml/minute, about 30-60 ml/minute, about 60-120 ml/minute, about 120-240 ml/minute, about 240-360 ml/minute, about 360-480 ml/minute, or about 480-600 ml/minute). In some embodiments, an mRNA stock solution is mixed at a flow rate of or greater than
5 about 5 ml/minute, 10 ml/minute, 15 ml/minute, 20 ml/minute, 25 ml/minute, 30 ml/minute, 35 ml/minute, 40 ml/minute, 45 ml/minute, 50 ml/minute, 60 ml/minute, 80 ml/minute, 100 ml/minute, 200 ml/minute, 300 ml/minute, 400 ml/minute, 500 ml/minute, or 600 ml/minute.

The process of incorporation of a desired mRNA into a lipid nanoparticle is referred to as “loading.” Exemplary methods are described in Lasic et al., *FEBS Lett.* (1992) 312:255-8. The LNP-incorporated
10 nucleic acids may be completely or partially located in the interior space of the lipid nanoparticle, within the bilayer membrane of the lipid nanoparticle, or associated with the exterior surface of the lipid nanoparticle membrane. The incorporation of an mRNA into lipid nanoparticles is also referred to herein as “encapsulation” wherein the nucleic acid is entirely or substantially contained within the interior space of the lipid nanoparticle.

15 Suitable LNPs may be made in various sizes. In some embodiments, decreased size of lipid nanoparticles is associated with more efficient delivery of an mRNA. Selection of an appropriate LNP size may take into consideration the site of the target cell or tissue and to some extent the application for which the lipid nanoparticle is being made.

A variety of methods known in the art are available for sizing of a population of lipid nanoparticles. Preferred methods herein utilize Zetasizer Nano ZS (Malvern Panalytical) to measure LNP particle size. In
20 one protocol, 10 µl of an LNP sample are mixed with 990 µl of 10% trehalose. This solution is loaded into a cuvette and then put into the Zetasizer machine. The z-average diameter (nm), or cumulants mean, is regarded as the average size for the LNPs in the sample. The Zetasizer machine can also be used to measure the polydispersity index (PDI) by using dynamic light scattering (DLS) and cumulant analysis of the
25 autocorrelation function. Average LNP diameter may be reduced by sonication of formed LNP. Intermittent sonication cycles may be alternated with quasi-elastic light scattering (QELS) assessment to guide efficient lipid nanoparticle synthesis.

In some embodiments, the majority of purified LNPs, i.e., greater than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the LNPs, have a size of about 70-150 nm (e.g.,
30 about 145 nm, about 140 nm, about 135 nm, about 130 nm, about 125 nm, about 120 nm, about 115 nm, about 110 nm, about 105 nm, about 100 nm, about 95 nm, about 90 nm, about 85 nm, or about 80 nm). In some embodiments, substantially all (e.g., greater than 80 or 90%) of the purified lipid nanoparticles have a size of about 70-150 nm (e.g., about 145 nm, about 140 nm, about 135 nm, about 130 nm, about 125 nm, about 120 nm, about 115 nm, about 110 nm, about 105 nm, about 100 nm, about 95 nm, about 90 nm, about
35 85 nm, or about 80 nm).

In some embodiments, the LNPs in the present composition have an average size of less than 150 nm, less than 120 nm, less than 100 nm, less than 90 nm, less than 80 nm, less than 70 nm, less than 60 nm, less than 50 nm, less than 30 nm, or less than 20 nm.

5 In some embodiments, greater than about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% of the LNPs in the present composition have a size ranging from about 40-90 nm (*e.g.*, about 45-85 nm, about 50-80 nm, about 55-75 nm, about 60-70 nm) or about 50-70 nm (*e.g.*, 55-65 nm) are particular suitable for pulmonary delivery via nebulization.

10 In some embodiments, the dispersity, or measure of heterogeneity in size of molecules (PDI), of LNPs in a pharmaceutical composition provided by the present disclosure is less than about 0.5. In some embodiments, an LNP has a PDI of less than about 0.5, less than about 0.4, less than about 0.3, less than about 0.28, less than about 0.25, less than about 0.23, less than about 0.20, less than about 0.18, less than about 0.16, less than about 0.14, less than about 0.12, less than about 0.10, or less than about 0.08. The PDI may be measured by a Zetasizer machine as described above.

15 In some embodiments, greater than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the purified LNPs in a pharmaceutical composition provided herein encapsulate an mRNA within each individual particle. In some embodiments, substantially all (*e.g.*, greater than 80% or 90%) of the purified lipid nanoparticles in a pharmaceutical composition encapsulate an mRNA within each individual particle. In some embodiments, a lipid nanoparticle has an encapsulation efficiency of between 50% and 99%; or greater than about 60, 65, 70, 75, 80, 85, 90, 92, 95, 98, or 99%. Typically, lipid nanoparticles for use
20 herein have an encapsulation efficiency of at least 90% (*e.g.*, at least 91, 92, 93, 94, or 95%).

In some embodiments, an LNP has a N/P ratio of between 1 and 10. In some embodiments, a lipid nanoparticle has a N/P ratio above 1, about 1, about 2, about 3, about 4, about 5, about 6, about 7, or about 8. In further embodiments, a typical LNP herein has an N/P ratio of 4.

25 In some embodiments, a pharmaceutical composition according to the present disclosure contains at least about 0.5 µg, 1 µg, 5 µg, 10 µg, 100 µg, 500 µg, or 1000 µg of encapsulated mRNA. In some embodiments, a pharmaceutical composition contains about 0.1 µg to 1000 µg, at least about 0.5 µg, at least about 0.8 µg, at least about 1 µg, at least about 5 µg, at least about 8 µg, at least about 10 µg, at least about 50 µg, at least about 100 µg, at least about 500 µg, or at least about 1000 µg of encapsulated mRNA.

30 In some embodiments, mRNA can be made by chemical synthesis or by *in vitro* transcription (IVT) of a DNA template. In this process, in an IVT process, a DNA template, such as a cDNA or pDNA template is used to produce an mRNA transcript and the DNA template is degraded by a DNase. The transcript is purified by depth filtration and tangential flow filtration (TFF). The purified transcript is further modified by adding a cap and a tail, and the modified RNA is purified again by depth filtration and TFF.

The mRNA is then prepared in an aqueous buffer and mixed with an amphiphilic solution containing the lipid components of the LNPs. An amphiphilic solution for dissolving the four lipid components of the LNPs may be an alcohol solution. In some embodiments, the alcohol is ethanol. The aqueous buffer may be, for example, a citrate, phosphate, acetate, or succinate buffer and may have a pH of about 3.0-7.0, e.g., about 3.5, about 4.0, about 4.5, about 5.0, about 5.5, about 6.0, or about 6.5. The buffer may contain other components such as a salt (e.g., sodium, potassium, and/or calcium salts). In particular embodiments, the aqueous buffer has 1 mM citrate, 150 mM NaCl, pH 3.5 or 4.5.

An exemplary process for making an mRNA-LNP composition involves mixing of a buffered mRNA solution with a solution of lipids in ethanol in a controlled homogeneous manner, where the ratio of lipids:mRNA is maintained throughout the mixing process. In this illustrative example, the mRNA is presented in an aqueous buffer containing citric acid monohydrate, tri-sodium citrate dihydrate, and sodium chloride. The mRNA solution is added to the solution (1 mM citrate buffer, 150 mM NaCl, pH 4.5). The lipid mixture of four lipids (e.g., a cationic lipid, a PEGylated lipid, a cholesterol-based lipid, and a helper lipid) is dissolved in ethanol. The aqueous mRNA solution and the ethanol lipid solution are mixed at a volume ratio of 4:1 in a “T” mixer with a near “pulseless” pump system. The resultant mixture is then subjected for downstream purification and buffer exchange. The buffer exchange may be achieved using dialysis cassettes or a TFF system. TFF may be used to concentrate and buffer-exchange the resulting nascent LNP immediately after formation via the T-mix process. The diafiltration process is a continuous operation, keeping the volume constant by adding appropriate buffer at the same rate as the permeate flow.

Vectors

In one aspect, disclosed herein are vectors comprising a nucleic acid disclosed herein. In some embodiments, mRNAs as described herein may be cloned into a vector. Vectors include, but are not limited to, a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors also include expression vectors, replication vectors, probe generation vectors, sequencing vectors, and vectors optimized for in vitro transcription (IVT).

In certain embodiments, the vector can be used to express mRNA in a host cell. In various embodiments, the vector can be used as a template for IVT. The construction of optimally translated IVT mRNA suitable for therapeutic use is disclosed in detail in Sahin, et al. (2014). Nat. Rev. Drug Discov. 13, 759–780; Weissman (2015). Expert Rev. Vaccines 14, 265–281.

In some embodiments, the vectors disclosed herein can comprise at least the following, from 5' to 3': an RNA polymerase promoter; a polynucleotide sequence encoding a 5' UTR; a polynucleotide sequence encoding an ORF; a polynucleotide sequence encoding a 3' UTR; and a polynucleotide sequence encoding at least one RNA aptamer. In some embodiments, the vectors disclosed herein may comprise a polynucleotide sequence encoding a poly(A) sequence and/or a polyadenylation signal.

A variety of RNA polymerase promoters are known. In some embodiments, the promoter can be a T7 RNA polymerase promoter. Other useful promoters can include, but are not limited to, T3 and SP6 RNA polymerase promoters. Consensus nucleotide sequences for T7, T3, and SP6 promoters are known.

Also disclosed herein are host cells (e.g., mammalian cells, e.g., human cells) comprising the vectors or nucleic acids disclosed herein. A "host cell" includes an individual cell or cell culture which can be or has been a recipient of exogenous nucleic acid. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. Host cells include cells transfected or infected *in vivo* or *in vitro* with nucleic acid or vector disclosed herein.

Vectors can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaza Nucleofactor-II (Amaza Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendorf, Hamburg, Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, biolistic particle delivery systems such as "gene guns" (see, for example, Nishikawa, et al. (2001). Hum Gene Ther. 12(8):861-70, or the TransIT-RNA transfection Kit (Mirus, Madison, WI).

Chemical means for introducing a vector into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (e.g., an artificial membrane vesicle).

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present disclosure, in order to confirm the presence of the mRNA sequence in the host cell a variety of assays may be performed.

Self-Replicating RNA, Trans-Replicating RNA and Non-Replicating RNA

Typically, the nucleic acid molecules described herein are non-replicating RNAs. However, the nucleic acid molecules described herein may alternatively be self-replicating RNAs or trans-replicating RNAs.

Self-replicating RNA

Self-replicating (or self-amplifying) RNA can be produced by using replication elements derived from, e.g., alphaviruses, and substituting the structural viral proteins with a nucleotide sequence encoding a protein of interest (e.g., a *Chlamydia sp.* antigen). A self-replicating RNA is typically a positive-strand molecule which can be directly translated after delivery to a cell, and this translation provides an RNA-dependent RNA polymerase which then produces both antisense and sense transcripts from the delivered RNA. Thus, the delivered RNA leads to the production of multiple daughter RNAs. These daughter RNAs, as well as

collinear subgenomic transcripts, may be translated themselves to provide in situ expression of an encoded antigen, or may be transcribed to provide further transcripts with the same sense as the delivered RNA which are translated to provide in situ expression of the antigen. The overall result of this sequence of transcriptions is a large amplification in the number of the introduced replicon RNAs and so the encoded antigen becomes a major polypeptide product of the cells.

One suitable system for achieving self-replication in this manner is to use an alphavirus-based replicon. These replicons are positive stranded (positive sense-stranded) RNAs which lead to translation of a replicase (or replicase-transcriptase) after delivery to a cell. The replicase is translated as a polyprotein which auto-cleaves to provide a replication complex which creates genomic-strand copies of the positive-strand delivered RNA. These negative (-)-stranded transcripts can themselves be transcribed to give further copies of the positive-stranded parent RNA and also to give a subgenomic transcript which encodes the antigen. Translation of the subgenomic transcript thus leads to in situ expression of the antigen by the infected cell. Suitable alphavirus replicons can use a replicase from a Sindbis virus, a Semliki forest virus, an eastern equine encephalitis virus, a Venezuelan equine encephalitis virus, etc. Mutant or wild-type virus sequences can be used, e.g., the attenuated TC83 mutant of VEEV has been used in replicons, see the following reference: WO2005/113782, incorporated herein by reference.

In one embodiment, each self-replicating RNA described herein encodes (i) an RNA-dependent RNA polymerase which can transcribe RNA from the self-replicating RNA molecule and (ii) a *Chlamydia sp.* antigen. The polymerase can be an alphavirus replicase, e.g., comprising one or more of alphavirus proteins nsP1, nsP2, nsP3, and nsP4. Whereas natural alphavirus genomes encode structural virion proteins in addition to the non-structural replicase polyprotein, in certain embodiments, the self-replicating RNA molecules do not encode alphavirus structural proteins. Thus, the self-replicating RNA can lead to the production of genomic RNA copies of itself in a cell, but not to the production of RNA-containing virions. The inability to produce these virions means that, unlike a wild-type alphavirus, the self-replicating RNA molecule cannot perpetuate itself in infectious form. The alphavirus structural proteins which are necessary for perpetuation in wild-type viruses are absent from self-replicating RNAs of the present disclosure and their place is taken by gene(s) encoding the immunogen of interest, such that the subgenomic transcript encodes the immunogen rather than the structural alphavirus virion proteins. Self-replicating RNA are described in further detail in WO2011005799, incorporated herein by reference.

Trans-Replicating RNA

Trans-replicating (or trans-amplifying) RNA possess similar elements as the self-replicating RNA described above. However, with trans replicating RNA, two separate RNA molecules are used. A first RNA molecule encodes for the RNA replicase described above (e.g., the alphavirus replicase) and a second RNA molecule encodes for the protein of interest (e.g., a *Chlamydia sp.* antigen described herein). The RNA replicase may replicate one or both of the first and second RNA molecule, thereby greatly increasing the

copy number of RNA molecules encoding the protein of interest. Trans replicating RNA are described in further detail in WO2017162265, incorporated herein by reference.

Non-Replicating RNA

Non-replicating (or non-amplifying) RNA is an RNA without the ability to replicate itself.

5 **Therapeutic uses**

In another aspect, the invention provides the polypeptides, nucleic acids, combinations or compositions of the present invention for use as a medicament. The invention also provides the use of the polypeptides, nucleic acids, combinations or compositions of the present invention for the manufacture of a medicament. The medicament may be used for treating or preventing a disease as described herein. The invention further
10 provides a method of treating or preventing a disease comprising administering the polypeptides, nucleic acids, combinations or compositions of the present invention to a subject in need thereof. The polypeptides, nucleic acids, combinations or compositions of the present invention may, for example, be administered in an amount effective to treat or prevent the disease in the subject. Polypeptides, nucleic acids, combinations or compositions may thus be administered in an effective amount. Typically, the treatment is prophylactic.

15 In another aspect, the invention provides the polypeptides, nucleic acids, combinations or compositions of the present invention for use in treating or preventing a *Chlamydia sp.* infection in a subject (e.g., human). The invention also provides the use of the polypeptides, nucleic acids, combinations or compositions of the present invention for the manufacture of a medicament for treating or preventing a *Chlamydia sp.* infection in a subject (e.g., human). The invention further provides a method of treating or preventing a *Chlamydia*
20 *sp.* infection in a subject (e.g., human), the method comprising administering the polypeptides, nucleic acids, combinations or compositions of the present invention to the subject. The polypeptides, nucleic acids, combinations or compositions of the present invention may, for example, be administered in an amount effective to treat or prevent a *Chlamydia sp.* infection in the subject (i.e. administered in an effective amount). The polypeptides, nucleic acids, combinations or compositions may be used for generating an
25 immune response against *Chlamydia sp.* infection in a subject (e.g., human). In preferred embodiments, the infection is a *C. trachomatis* infection.

The invention provides the polypeptides, nucleic acids, combinations or compositions of the present invention for use in treating or preventing trachoma (a disease of the eye caused by *C. trachomatis* infection), genital tract infection by *Chlamydia trachomatis*, lymphogranuloma venereum (a disease caused
30 by *C. trachomatis*), oropharyngeal infection by *C. trachomatis* or a rectal infection by *C. trachomatis* in a subject (e.g., human). The invention also provides the use of the polypeptides, nucleic acids, combinations or compositions of the present invention for the manufacture of a medicament for treating or preventing trachoma caused by *C. trachomatis* infection, genital tract infection by *Chlamydia trachomatis*, lymphogranuloma venereum (a disease caused by *C. trachomatis*), oropharyngeal infection by *C.*

trachomatis or a rectal infection by *C. trachomatis* in a subject (e.g., human). The invention further provides a method of treating or preventing a trachoma caused by *C. trachomatis* infection, genital tract infection by *Chlamydia trachomatis*, lymphogranuloma venereum caused by *C. trachomatis* infection, oropharyngeal infection by *C. trachomatis* or a rectal infection by *C. trachomatis* in a subject (e.g., human), the method comprising administering the polypeptides, nucleic acids, combinations or compositions of the present invention to the subject. The polypeptides, nucleic acids, combinations or compositions of the present invention may, for example, be administered in an amount effective to treat or prevent a trachoma caused by *C. trachomatis* infection, genital tract infection by *Chlamydia trachomatis*, lymphogranuloma venereum caused by *C. trachomatis* infection, oropharyngeal infection by *C. trachomatis* or a rectal infection by *C. trachomatis* in the subject (i.e. administered in an effective amount). In preferred embodiments, the infection is a *C. trachomatis* infection. Trachoma may be caused by infection with *C. trachomatis* serovars A, B, Ba or C. Lymphogranuloma venereum may be caused by infection with *C. trachomatis* serovars L1, L2 and L3. Genital tract infection may be caused by serovars D-K. In preferred embodiments, the *Chlamydia sp.* (e.g., *C. trachomatis*) infection is a genital tract infection.

The invention provides the polypeptides, nucleic acids, combinations or compositions of the present invention for use in a method of providing protective immunity against a *Chlamydia sp.* infection in a subject (e.g., human). The invention also provides the use of the polypeptides, nucleic acids, combinations or compositions of the present invention for the manufacture of a medicament for use in a method of providing protective immunity against a *Chlamydia sp.* infection in a subject (e.g., human). The invention further provides a method of providing protective immunity against a *Chlamydia sp.* infection in a subject (e.g., human), the method comprising administering the polypeptides, nucleic acids, combinations or compositions of the present invention to the subject. The polypeptides, nucleic acids, combinations or compositions of the present invention may, for example, be administered in an amount effective to providing protective immunity against a *Chlamydia sp.* infection in the subject (i.e. administered in an effective amount). In preferred embodiments, the infection is a *C. trachomatis* infection.

The polypeptides, nucleic acids, combinations or compositions of the invention may elicit a T cell response (e.g., an antigen-specific T cell response) in a subject. The polypeptides, nucleic acids, combinations or compositions of the invention may elicit a CD4+ T cell response and/or a CD8+ T cell response (e.g., CD4+ T cell response) in a subject. In some embodiments, the polypeptides, nucleic acids, combinations or compositions of the invention may elicit IFN γ -producing T cells such as IFN γ -producing CD4+ T cells and/or IFN γ -producing CD8+ T cells (e.g., IFN γ -producing CD4+ T cells) in a subject. In some embodiments, the polypeptides, nucleic acids, combinations or compositions of the invention may elicit CD4+ IFN γ +IL2+TNF α + T cells (i.e. CD4+ T cells which produce IFN γ , IL2 and TNF α). In some embodiments, the polypeptides, nucleic acids, combinations or compositions of the invention may elicit CD8+ IFN γ +IL2+TNF α + T cells or CD8+ T cells which produce IFN γ and TNF α). In some embodiments, the polypeptides, nucleic acids, combinations or compositions of the invention may elicit a cross-serovar T

cell immune response in a subject, i.e. a T cell immune response that is cross-reactive against two or more serovars of *Chlamydia sp.* (e.g., cross-reactive against two or more serovars of *C. trachomatis*). Cytokine production by T cells may be measured by intracellular cytokine staining of T cells, e.g., T cells obtained from a subject, or by detecting secreted cytokines (e.g., by ELISA). The polypeptides, nucleic acids, combinations or compositions of the invention may elicit a Th1 response (e.g., a Th1 T cell response) in a subject.

The polypeptides, nucleic acids, combinations or compositions of the invention may elicit an antibody (e.g., IgG) response in a subject, such as a neutralising antibody (IgG) response. The antibodies may be of any isotype (e.g., IgA, IgG, IgM i.e. an α , γ or μ heavy chain), but will generally be IgG. Within the IgG isotype, antibodies may be IgG1, IgG2, IgG3 or IgG4 subclass. The antibody may have a κ or a λ light chain. A “neutralising antibody” is an antibody which neutralises the biological effects of a *Chlamydia sp.* antigen in a subject. In some embodiments, the polypeptides, nucleic acids, combinations or compositions of the invention may elicit a cross-serovar antibody response in a subject, i.e. an antibody response that is cross-reactive against two or more serovars of *Chlamydia sp.* (e.g., cross-reactive against two or more serovars of *C. trachomatis*).

In some embodiments, the polypeptides, nucleic acids, combinations or compositions of the invention may elicit a T cell response as described herein and/or an antibody response as described herein.

Administration of a polypeptide, nucleic acid, combination or composition of the invention to a subject may enable the subject to produce a *Chlamydia sp.* antigen-responsive CD4 memory T cell population or CD8 memory T cell population (e.g., CD4 T cell memory population) on exposure to *Chlamydia sp.* bacteria or the *Chlamydia sp.* antigen. The *Chlamydia sp.*-responsive CD4 memory T cell population may confer “sterilizing” immunity (i.e. complete protective immunity) against re-infection.

Administration of a polypeptide, nucleic acid, combination or composition of the invention to a subject may enable the subject to produce a *Chlamydia sp.* antigen-responsive memory B cell population on exposure to *Chlamydia sp.* bacteria or the *Chlamydia sp.* antigen. The *Chlamydia sp.*-responsive memory B cell population may confer “sterilizing” immunity (i.e. complete protective immunity) against re-infection.

The polypeptides, nucleic acids, combinations or compositions of the invention may be used to induce a primary immune response and/or to boost an immune response.

The polypeptides, nucleic acids, combinations or compositions of the invention may be administered to a subject to allow the subject to develop and/or maintain sterilising immunity to a *Chlamydia sp.* infection. Protective immunity may be provided or augmented in an immunised subject following a *Chlamydia sp.* infection.

The polypeptides, nucleic acids, combinations or compositions of the invention may be used in a prime-boost vaccination regime. Protective immunity against a *Chlamydia sp.* infection according to the invention may be provided by administering a priming vaccine, comprising a polypeptide, nucleic acid, combinations or composition of the invention, followed by a booster vaccine. The booster vaccine may be the same as the primer vaccine.

In certain embodiments, the subject is a vertebrate, *e.g.*, a mammal, such as a human or a veterinary mammal (*e.g.*, cat, dog, horse, cow, sheep, cattle, deer, goat, pig, rodents (*e.g.*, mice)). In preferred embodiments, the subject is a human. The subject (*e.g.*, the human subject) may be male or female. In some embodiments, human subjects may be 7-50 (*e.g.*, 9-44) years old.

Modes of administration

The polypeptides, nucleic acids, combinations or compositions of the present invention can be administered parenterally (*e.g.*, intramuscularly, intradermally, subcutaneously, intraperitoneally, intravenously, or to the interstitial space of a tissue) or by rectal, oral, vaginal, topical, transdermal, intranasal, sublingual, ocular, aural, pulmonary or other mucosal administration. In some embodiments, the compositions of the invention are administered intramuscularly. In some embodiments, the compositions of the invention are delivered by mucosal administration.

In certain embodiments, a polypeptide, nucleic acids, combinations or composition (*e.g.*, a composition) of the invention is provided for use in intramuscular (IM) injection. The polypeptide, nucleic acids, combinations or composition (*e.g.*, the composition) can be administered to the thigh or the upper arm of a subject at, *e.g.*, their deltoid muscle in the upper arm. In some embodiments, the polypeptide, nucleic acids, combinations or composition (*e.g.*, the composition) is provided in a pre-filled syringe or injector (*e.g.*, single-chambered or multi-chambered). Injection may be via a needle (*e.g.*, a hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dose is 0.5 ml. In some embodiments, the polypeptide, nucleic acids, combinations or composition (*e.g.*, the composition) is provided for use in inhalation and is provided in a pre-filled pump, aerosolizer, or inhaler.

In certain embodiments, a polypeptide, nucleic acids, combinations or composition (*e.g.*, a composition) of the invention is provided for use in skin injection, *e.g.*, in the epidermis, the dermis or the hypodermis of the skin. In some embodiments, a composition is provided in a device suitable for skin injection, such as a needle (*e.g.*, an epidermic, dermic or hypodermic needle), a needle free device, a microneedle device or a microprojection array device. Examples of microneedle or microprojection array devices suitable for the skin injection according to the invention are described in US20230270842A1, US20220339416A1, US20210085598A1, US20200246450A1, US20220143376A1, US20180264244A1, US20180263641A1, US20110245776A1.

The compositions of the invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses (e.g., two or three) may be used in a primary immunisation schedule and/or in a booster immunisation schedule. A primary dose schedule may be followed by a booster dose schedule. Multiple doses (e.g., two doses or three) will typically be administered at least 1 week apart (e.g., about 2 weeks, about 3 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 10 weeks, about 12 weeks, about 16 weeks, etc.) to subjects in need thereof to achieve the desired therapeutic or prophylactic effects. The doses (e.g., prime and booster doses) may be separated by an interval of e.g., 1 week, 2 weeks, 3 weeks, 4 weeks, one month, two months, three months, four months, five months, six months, one year, two years, five years, or ten years

A composition of the invention may be in the form of an extemporaneous formulation, e.g., a composition of the invention may be lyophilised. Such compositions may be reconstituted with a physiological buffer (e.g., PBS) just before use. The compositions of the invention may be provided in the form of an aqueous solution or a frozen aqueous solution and can be directly administered to subjects without reconstitution (after thawing, if previously frozen).

In some embodiments of a composition comprising a mRNA as described herein, a single dose of the composition contains 1 to 400 µg, such as 1-50 µg, of a mRNA as described herein (e.g., monovalent or multivalent). For example, a single dose may contain about 2.5 µg, about 5 µg, about 7.5 µg, about 10 µg, about 12.5 µg, or about 15 µg of a mRNA described herein e.g., for intramuscular (IM) injection.

In some embodiments, the composition comprises two nucleic acids (e.g., two mRNAs) as described herein encoding different polypeptides as described herein and the nucleic acids is present in a weight ratio of 1:1. In some embodiments, the composition comprises three nucleic acids (e.g., three mRNAs) as described herein encoding different polypeptides as described herein and the nucleic acids are present in a weight ratio of 1:1:1. Typically, the LNPs described herein co-encapsulate (a) a nucleic acid as described herein comprising a nucleotide sequence encoding a modified MOMP polypeptide as described herein, (b) a nucleic acid as described herein comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide as described herein, (c) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide as described herein, and (d) a nucleic acid as described herein comprising a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide as described herein. Typically, the composition comprises four nucleic acids (e.g., four mRNAs) as described herein encoding different polypeptides as described herein. Typically the nucleic acids are present in a weight ratio of 1:1:1:1.

In further embodiments, a composition of the invention may be provided as a multi-valent single dose contains multiple (e.g., 2, 3, or 4) kinds of LNPs, each for a different antigen, and each kind of LNP has an mRNA amount of, e.g., 2.5 µg, about 5 µg, about 7.5 µg, about 10 µg, about 12.5 µg, or about 15 µg.

In some embodiments, the subject is administered one or more nucleic acid compositions of the present invention. The nucleic acid compositions may comprise a nucleic acid comprising a nucleotide sequence encoding a polypeptide antigen as described herein. The nucleic acid compositions may be administered simultaneously, separately or sequentially. In some embodiments, the subject is administered a nucleic acid combination of the present invention. The nucleic acid combinations include combinations of two or more nucleic acids as described herein. The nucleic acids within a combination may be administered simultaneously, separately or sequentially.

In some embodiments, the subject is administered one or more polypeptide compositions of the present invention. The polypeptide compositions may comprise a polypeptide antigen as described herein. The polypeptide compositions may be administered simultaneously, separately or sequentially. In some embodiments, the subject is administered a polypeptide combination of the present invention. The polypeptide combinations include combinations of two or more polypeptides as described herein. The nucleic acids within a combination may be administered simultaneously, separately or sequentially

In some embodiments, the subject is administered one or more nucleic acid compositions of the present invention and one or more polypeptide compositions of the invention. In some embodiments, the subject is administered a nucleic acid composition comprising a nucleotide sequence encoding a modified MOMP polypeptide (and optionally a nucleic acid composition comprising a nucleotide sequence encoding a chimeric MOMP VD polypeptide) and a polypeptide composition comprising one more polypeptides comprising a polypeptide sequence of a non-MOMP antigen. In some embodiments, the subject is administered two or more polypeptide compositions each comprising a polypeptide comprising a polypeptide sequence of a non-MOMP antigen. The nucleic acid composition and the one or more polypeptide compositions may be administered simultaneously, separately or sequentially.

Compositions administered separately or sequentially may be administered within 12 months of each other, within six months of each other, or within one month or less of each other (e.g., within 10 days). Compositions may be administered within 7 days, within 3 days, within 2 days, or within 24 hours of each other. Simultaneous administration may involve administering the compositions of the invention at the same time. Simultaneous administration may include administration of the compositions of the invention to a patient within 12 hours of each other, within 6 hours, within 3 hours, within 2 hours or within 1 hour of each other, typically within the same visit to a clinical centre.

The present invention also provides a kit comprising one or more compositions described herein in one or more containers or provides one or more composition as described herein in one or more containers and a physiological buffer for reconstitution in another container. The container(s) may contain a single-use dosage or multi-use dosage. The containers may be pre-treated glass vials or ampules. The kit may include instructions for use.

A method for the detection and quantification of antibodies against one or more of the polypeptides described herein in a serum sample is also provided herein. For example, antibodies against MOMP, CT443, and/or CT584 polypeptides may be detected and quantified. Detection and quantification of antibodies against each polypeptide may be performed separately or simultaneously, e.g., as a panel in a single multi-well plate. Thus, a multi-well plate comprising wells coated with each of the recombinant polypeptides MOMP, CT443, and CT584, is further provided herein.

Definitions

The term “comprising” encompasses “including” as well as “consisting” e.g., a composition “comprising” X may consist exclusively of X or may include something additional e.g., X + Y.

The term “about” in relation to a numerical value x is optional and means, for example, $x \pm 10\%$.

The term “a” or “an” entity refers to one or more of that entity; for example, “a nucleotide sequence” is understood to represent one or more nucleotide sequences. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

Furthermore, “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A” (alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

The term “*Chlamydia sp.*” as used herein refers to species of the genus *Chlamydia*, which comprises the species *Chlamydia trachomatis*, *Chlamydia abortus*, *Chlamydia pneumoniae*, *Chlamydia muridarum*, *Chlamydia psittaci*, *Chlamydia pecorum*, *Chlamydia felis*, and *Chlamydia caviae*. In preferred embodiments of the invention, the *Chlamydia sp.* is *C. trachomatis*.

As used herein, the term “effective amount” refers to an amount (e.g., of a nucleic acid, a polypeptide, a combination or a composition as described herein) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages, and is not intended to be limited to a particular formulation or administration route. The term “effective amount” includes, e.g., therapeutically effective amount and/or prophylactically effective amount. The term “effective amount” as used herein refers to an amount (e.g., of a nucleic acid, a polypeptide, a combination or a composition as described herein) which is effective for producing some desired therapeutic or prophylactic effects in the treatment or prevention of an infection, disease, disorder and/or condition at a reasonable benefit/risk ratio applicable to any medical treatment.

The term “elementary body” refers to one of the forms of *Chlamydia sp.* bacteria. Elementary bodies may be released from infected cells and can be transmitted from one subject to another.

5 The term "fragment" or "variant" when referring to the polypeptides of the present disclosure include any polypeptides which retain at least some of the properties (e.g., specific antigenic property of the polypeptide or the ability of polypeptide to contribute to the induction of antibody binding) of the reference polypeptide. Fragments of polypeptides include N-terminally and/or C-terminally truncated fragments, e.g., C-terminal fragments and N-terminal fragments, as well as deletion fragments but do not include the naturally occurring full-length polypeptide (or mature polypeptide). A deletion fragment refers to a polypeptide with 1 or more internal amino acids deleted from the full-length polypeptide. Variants of polypeptides include 10 fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants can be naturally or non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions. Such variations (i.e. truncations and/or amino acid substitutions, deletions, or insertions) may occur either on the amino 15 acid level or correspondingly on the nucleic acid level.

Identity with respect to a sequence is defined herein as the percentage of nucleic acid or amino acid residues in the candidate sequence that are identical with the reference amino acid sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

20 Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides or the nucleic acids of two polynucleotides. For example, using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., 25 in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. The percent identity can be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the shorter sequences in order to align the two sequences.

30 As used herein, the term “kit” refers to a packaged set of related components, such as one or more compounds or compositions and one or more related materials such as solvents, solutions, buffers, instructions, or desiccants.

The term “linked” or “attached” as used herein refers to a first amino acid sequence or nucleotide sequence covalently joined to a second amino acid sequence or nucleotide sequence, respectively (e.g., a secretion

signal peptide amino acid sequence and/or a heterologous transmembrane domain amino acid sequence linked to a *Chlamydia sp.* polypeptide amino acid sequence). The first amino acid or nucleotide sequence can be directly joined to the second amino acid or nucleotide sequence or alternatively an intervening sequence can covalently join the first sequence to the second sequence. The term “linked” means not only a fusion of a first amino acid sequence to a second amino acid sequence at the C-terminus or the N-terminus, but also includes insertion of the whole first amino acid sequence (or the second amino acid sequence) into any two amino acids in the second amino acid sequence (or the first amino acid sequence, respectively). In one embodiment, the first amino acid sequence can be linked to a second amino acid sequence by a peptide bond or a linker. The first nucleotide sequence can be linked to a second nucleotide sequence by a phosphodiester bond or a linker. The linker can be a peptide or a polypeptide (for polypeptide chains) or a nucleotide or a nucleotide chain (for nucleotide chains) or any chemical moiety (for both polypeptide and polynucleotide chains). The term “linked” is also indicated by a hyphen (-).

The term “native” as used herein refers to naturally occurring sequences. For example, a native *Chlamydia sp.* MOMP polypeptide is a naturally occurring *Chlamydia sp.* MOMP polypeptide.

Embodiments

The invention includes at least the following numbered embodiments:

1. A nucleic acid comprising a nucleotide sequence encoding a modified Major Outer Membrane Protein (MOMP) polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences.
2. The nucleic acid of embodiment 1, wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.
3. The nucleic acid of embodiment 1 or 2, wherein the modified MOMP polypeptide does not comprise a native *Chlamydia sp.* MOMP variable domain between the two or more conserved domain sequences.
4. The nucleic acid of any one of embodiments 1-3, wherein the modified MOMP polypeptide comprises five conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein
 - (i) the modified MOMP polypeptide comprises all five full-length conserved domains of a native *Chlamydia sp.* MOMP polypeptide;
 - (ii) the conserved domain sequences of the modified MOMP polypeptide is least 95% identical to a conserved domain of a native MOMP polypeptide of a *Chlamydia sp.* of any serovar (e.g., a conserved domain as defined in Table 1);

- (iii) the conserved domains of the modified MOMP polypeptide collectively have at least 95% sequence identity to the conserved domains of a native MOMP polypeptide (e.g., serovar E MOMP); and/or
- (iv) the conserved domain sequences of the modified MOMP polypeptide lack up to 3 or 5 amino acids of a native *Chlamydia sp.* MOMP conserved domain sequence.
- 5
5. The nucleic acid of any one of embodiments 1-4, wherein
- (i) the modified MOMP polypeptide comprises a non-native loop sequence between each of the conserved domain sequences;
- (ii) the non-native loop sequence is no more than 40% identical to any native *Chlamydia sp.* MOMP VD (VD1, VD2, VD3 or VD4) sequences of any serovar (e.g., serovars D, E, F or G of *C. trachomatis*).
- 10
6. The nucleic acid of embodiment 4, wherein the modified MOMP polypeptide comprises four non-native loop sequences and does not comprise any native *Chlamydia sp.* MOMP variable domains between the conserved domain sequences.
- 15
7. The nucleic acid of any one of embodiments 1-6, wherein the modified MOMP polypeptide does not comprise any native *Chlamydia sp.* MOMP variable domains between any of the conserved domain sequences.
8. The nucleic acid of any one of embodiments 1-7, wherein
- (i) the non-native loop sequence is between 3 and 30 amino acids in length, e.g., between 4 and 20 amino acids in length; and/or
- 20
- (ii) the two or more conserved domain sequences are linked by a non-native loop sequence such that the conserved domain sequences form a beta-barrel structure (e.g., made up of anti-parallel beta strands), optionally wherein the beta-barrel structure is as predicted *in silico* (e.g., using Alphafold2 (Deepmind) software).
- 25
9. The nucleic acid of any one of embodiments 1-8, wherein
- (i) a non-native loop sequence replacing VD1 comprises a sequence according to SEQ ID NO: 462 or 466 (e.g., SEQ ID NO: 462);
- (ii) a non-native loop sequence replacing VD2 comprises a sequence according to SEQ ID NO: 463 or 467 (e.g., SEQ ID NO: 463);
- 30
- (iii) a non-native loop sequence replacing VD3 comprises a sequence according to SEQ ID NOs 464 or 468 (e.g., SEQ ID NO: 464); and/or

- (iv) a non-native loop sequence replacing VD4 comprises a sequence according to SEQ ID NO: 465 or 469 (e.g., SEQ ID NO: 465),

e.g., wherein the modified MOMP polypeptide comprises four non-native loop sequences according to SEQ ID NOs 462, 463, 464 and 465 in place of VD1, VD2, VD3 and VD4, respectively.

5

10. The nucleic acid of any one of embodiments 1-9, wherein the modified MOMP polypeptide comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the single amino acid substitution is a substitution of cysteine with serine.
- 10 11. The nucleic acid of any one of embodiments 1-10, wherein the modified MOMP polypeptide comprises a mutation at one or more (e.g., all) positions corresponding to a glycosylation site, optionally an N-glycosylation site, in a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the mutation is a single amino acid substitution.
12. The nucleic acid of any one of embodiments 1-11, wherein the modified MOMP polypeptide further comprises a secretion signal peptide sequence.
- 15 13. The nucleic acid of embodiment 12, wherein the secretion signal peptide sequence is a viral secretion signal peptide sequence, optionally selected from the group consisting of: an influenza hemagglutinin (HA) secretion signal peptide sequence, a SARS CoV-2 spike secretion signal peptide sequence, a VZV gB secretion signal peptide sequence, a VZV gE secretion signal peptide sequence, a VZV gI secretion signal peptide sequence, a VZV gK secretion signal peptide sequence, a measles F-protein secretion signal peptide sequence, a rubella E1 protein secretion signal peptide sequence, a rubella E2 protein secretion signal peptide sequence, a mumps F-protein secretion signal peptide sequence, an Ebola GP protein secretion signal peptide sequence, and a smallpox 6kDa IC protein secretion signal peptide sequence, optionally wherein the secretion signal peptide sequence comprises an amino acid sequence according to one of the SEQ ID NOs in Table 2 or Table 2.1.
- 20 25 14. The nucleic acid of embodiment 13, wherein the secretion signal peptide sequence comprises a secretion signal peptide sequence of HA protein of influenza A virus, e.g., wherein the secretion signal peptide sequence comprises a sequence according to SEQ ID NO: 187 or SEQ ID NO: 188.
- 30 15. The nucleic acid of any one of embodiments 1-14, wherein the modified MOMP polypeptide comprises a sequence according to any one of SEQ ID NOs: 486-489 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto, e.g., wherein the modified MOMP polypeptide

comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto.

16. The nucleic acid of any one of embodiments 1-15, wherein the nucleic acid comprises a nucleotide sequence according to any one of SEQ ID NOs: 551-566 or a sequence that has at least 50% identity thereto, e.g., wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto.

17. The nucleic acid of any one of embodiments 1-16, wherein the nucleic acid is a messenger RNA (mRNA), optionally wherein

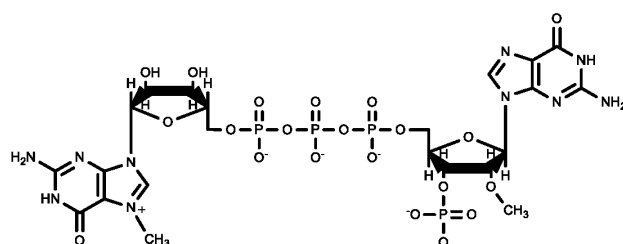
(i) the mRNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (poly(A)) sequence;

(ii) the mRNA is unmodified or comprises at least one chemical modification, optionally wherein the mRNA comprises at least one chemical modification, e.g., wherein the chemical modification comprises N1-methylpseudouridine; and/or

(iii) the mRNA is a self-replicating mRNA or a non-replicating mRNA, e.g., a non-replicating mRNA.

18. The nucleic acid of embodiment 17, wherein the mRNA comprises or consists of (e.g., consisting of) the following structural elements:

- a 5' cap, e.g., a cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;

- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 213;

- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and

- a polyA tail.

19. The nucleic acid of embodiment 18, wherein the mRNA is chemically modified and wherein the chemical modification comprises or consists of (e.g., consists of) N1-methylpseudouridine in place of every uridine.
- 5 20. A modified Major Outer Membrane Protein (MOMP) polypeptide having an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences.
21. The modified MOMP polypeptide of embodiment 20, wherein the modified MOMP polypeptide does not comprise a native *Chlamydia sp.* MOMP variable domain between the two or more conserved domain sequences.
- 10 22. The modified MOMP polypeptide of embodiment 20 or 21, wherein the modified MOMP polypeptide comprises five conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein (i) the modified MOMP polypeptide comprises all five full-length conserved domains of a native *Chlamydia sp.* MOMP polypeptide; and/or (ii) the conserved domains of the modified MOMP polypeptide collectively have at least 95% sequence identity to the conserved domains of a native MOMP polypeptide (e.g., serovar E MOMP).
- 15 23. The modified MOMP polypeptide of any one of embodiments 20-22, wherein the modified MOMP polypeptide comprises a non-native loop sequence between each of the conserved domain sequences.
24. The modified MOMP polypeptide of embodiment 22, wherein the modified MOMP polypeptide comprises four non-native loop sequences and does not comprise native *Chlamydia sp.* MOMP variable domains between the conserved domain sequences.
- 20 25. The modified MOMP polypeptide of any one of embodiments 20-24, wherein the modified MOMP polypeptide does not comprise any native *Chlamydia sp.* MOMP variable domains between any of the conserved domain sequences.
- 25 26. The modified MOMP polypeptide of any one of embodiments 20-25, wherein the non-native loop sequence is between 3 and 30 amino acids in length, e.g., between 4 and 20 amino acids in length.
27. The modified MOMP polypeptide of any one of embodiments 20-26, wherein
- (i) a non-native loop sequence replacing VD1 comprises a sequence according to SEQ ID NO: 462 or 466 (e.g., SEQ ID NO: 462);
- 30 (ii) a non-native loop sequence replacing VD2 comprises a sequence according to SEQ ID NO: 463 or 467 (e.g., SEQ ID NO: 463);

- (iii) a non-native loop sequence replacing VD3 comprises a sequence according to SEQ ID NOs 464 or 468 (e.g., SEQ ID NO: 464); and/or
 - (iv) a non-native loop sequence replacing VD4 comprises a sequence according to SEQ ID NO: 465 or 469 (e.g., SEQ ID NO: 465),
- 5 e.g., wherein the modified MOMP polypeptide may comprise four non-native loop sequences according to SEQ ID NOs 462-465 in place of VD1, VD2, VD3 and VD4, respectively.
28. The modified MOMP polypeptide of any one of embodiments 20-27, wherein the modified MOMP polypeptide comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the single amino acid substitution is a substitution of
- 10 cysteine with serine.
29. The modified MOMP polypeptide of any one of embodiments 20-28, wherein the modified MOMP polypeptide comprises a mutation at one or more (e.g., all) positions corresponding to an N-glycosylation site in a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the mutation
- 15 is a single amino acid substitution.
30. The modified MOMP polypeptide of any one of embodiments 20-29, wherein the modified MOMP polypeptide comprises a sequence according to any one of SEQ ID NO: 486-489 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto, e.g., wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least
- 20 70% (e.g., at least 90 or 95%) identity thereto.
31. A composition comprising the nucleic acid of any one of embodiments 1-19, preferably wherein the composition is an immunogenic composition.
32. A composition comprising the polypeptide of any one of embodiments 20-30, preferably wherein the composition is an immunogenic composition.
- 25 33. The composition of embodiment 31, wherein the composition further comprises:
- (i) a nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*; and/or
- 30 (ii) one or more (e.g., one, two, three, or four) of:
- (a) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide;

- (b) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide;
- (c) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide; and
- 5 (d) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide.
34. The composition of embodiment 33, wherein the composition comprises
- the nucleic acid of (i) and the nucleic acid of (ii)(a);
- the nucleic acid of (i) and the nucleic acid of (ii)(b); or
- 10 the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b);
- e.g., wherein the composition comprises the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b).
35. The composition of embodiment 33 or 34, wherein one or more nucleic acids is a mRNA, optionally wherein
- 15 (i) the mRNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (poly(A)) sequence;
- (ii) the mRNA is unmodified or comprises at least one chemical modification, optionally wherein the mRNA comprises at least one chemical modification e.g., wherein the chemical modification comprises N1-methylpseudouridine; and/or
- 20 (iii) the mRNA is a self-replicating mRNA or a non-replicating mRNA, e.g., a non-replicating mRNA.
36. The composition of any one of embodiments 31 or 33-35, wherein the composition further comprises a lipid nanoparticle (LNP), optionally wherein the nucleic acid is encapsulated in the LNP.
- 25 37. The composition of embodiment 32, wherein the composition further comprises:
- (i) a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*; and/or
- (ii) one or more (e.g., one, two, three or four) of:

- (a) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT443 polypeptide;
- (b) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT584 polypeptide;
- 5 (c) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT600 polypeptide; or
- (d) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT812 polypeptide,
- e.g., wherein the composition comprises
- 10 the polypeptide of (i) and the polypeptide of (ii)(a); or
- the polypeptide of (i) and the polypeptide of (ii)(b); or
- the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),
- e.g., wherein the composition comprises the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b).
- 15 38. The composition of any one of embodiments 33-37, wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.
39. The composition of any one of embodiments 33-38, wherein the chimeric MOMP VD polypeptide comprises conserved domain sequence portions of a native *Chlamydia sp.* MOMP polypeptide flanking each of the two or more MOMP VD sequences.
- 20 40. The composition of any one of embodiments 33-39, wherein the chimeric MOMP VD polypeptide comprises MOMP VD sequences of four different serovars, optionally wherein the different serovars are selected from serovars D, E, F or G of *C. trachomatis*.
41. The composition of any one of embodiments 33-40, wherein the chimeric MOMP VD polypeptide comprises:
- 25 (i) two MOMP VD1 sequences of different serovars of the *Chlamydia sp.* and/or
- (ii) two MOMP VD2 sequences of different serovars of the *Chlamydia sp.*; and/or
- (iii) one MOMP VD3 sequence; and/or
- (iv) two MOMP VD4 sequences of different serovars of the *Chlamydia sp.*,
- optionally wherein the different serovars are selected from serovars D, E, F or G of *C. trachomatis*.

42. The composition of any one of embodiments 33-40, wherein the chimeric MOMP VD polypeptide comprises one MOMP VD sequence of serovar D or E of *C. trachomatis* and one MOMP VD sequence of serovar F or G of *C. trachomatis*.
43. The composition of embodiment 41 or 42, wherein the chimeric MOMP VD polypeptide comprises at least one (e.g., four) of (i)-(iv):
- 5 (i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, or a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F or G (e.g., a MOMP VD1 sequence of serovar E and a MOMP VD1 sequence of serovar G); and/or
- 10 (ii) a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, or a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G (e.g., a MOMP VD2 sequence of serovar D and a MOMP VD2 sequence of serovar G); and/or
- (iii) a MOMP VD3 sequence from serovar G or a VD3 sequence from serovar F (e.g., serovar F); and/or
- 15 (iv) a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G, or a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F (e.g., a MOMP VD4 sequence of serovar D and a MOMP VD4 sequence of serovar F),
- wherein the serovars D, E, F or G are of *C. trachomatis*.
44. The composition of embodiment 43, wherein the chimeric MOMP VD polypeptide comprises:
- 20 (i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, a MOMP VD3 sequence from serovar G, a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G; or
- 25 (ii) a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G, a MOMP VD3 sequence from serovar F, a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F,
- wherein the serovars D, E, F or G are of *C. trachomatis*,
- e.g., wherein the chimeric MOMP VD polypeptide comprises MOMP VD sequences according to (ii).

45. The composition of any one of embodiments 39-44, wherein the conserved domain sequence portions are portions of conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide flanking the VD in its native *Chlamydia sp.* MOMP polypeptide.
- 5 46. The composition of any one of embodiments 39-45, wherein each conserved domain sequence portion comprises between 3 and 30 amino acid residues of a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, wherein the between 3 and 30 amino acid residues are immediately adjacent to the VD sequence in its native *Chlamydia sp.* MOMP polypeptide.
- 10 47. The composition of any one of embodiments 33-36 or 38-46, wherein the composition comprises the nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP VD polypeptide and wherein the chimeric MOMP VD polypeptide comprises a secretion signal peptide sequence.
- 15 48. The composition of embodiment 47, wherein the secretion signal peptide sequence is a viral secretion signal peptide sequence, optionally selected from the group consisting of: an influenza hemagglutinin (HA) secretion signal peptide sequence, a SARS CoV-2 spike secretion signal peptide sequence, a VZV gB secretion signal peptide sequence, a VZV gE secretion signal peptide sequence, a VZV gI secretion signal peptide sequence, a VZV gK secretion signal peptide sequence, a measles F-protein secretion signal peptide sequence, a rubella E1 protein secretion signal peptide sequence, a rubella E2 protein secretion signal peptide sequence, a mumps F-protein secretion signal peptide sequence, an Ebola GP protein secretion signal peptide sequence, and a smallpox 6kDa IC protein secretion signal peptide sequence, optionally wherein the secretion signal peptide sequence comprises an amino acid sequence according to any one of the SEQ ID NOs in Table 2 or Table 2.1.
- 20 49. The composition of embodiment 48, wherein the secretion signal peptide sequence comprises a secretion signal peptide sequence of HA protein of influenza A virus, *e.g.*, wherein the secretion signal peptide sequence comprises a sequence according to SEQ ID NO: 187 or SEQ ID NO: 188.
- 25 50. The composition of any one of embodiments 33-49, wherein the chimeric MOMP VD polypeptide comprises a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the single amino acid substitution is a substitution of cysteine with serine.
- 30 51. The composition of any one of embodiments 33-50, wherein the chimeric MOMP VD polypeptide comprises a mutation at one or more (*e.g.*, all) positions corresponding to a glycosylation site, optionally an N-glycosylation site, in a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the mutation is a single amino acid substitution.

52. The composition of embodiment 51, wherein the chimeric MOMP VD polypeptide comprises a single amino acid substitution at each of the amino acid residues corresponding to position 9 of SEQ ID NO: 9 (e.g., N to A substitution), position 11 of SEQ ID NO: 17 (e.g., T to A substitution), position 17 of SEQ ID NO: 6 (e.g., S to A substitution), positions 4 and 21 of SEQ ID NO: 18 (e.g., N to A and S to A substitutions, respectively), position 14 of SEQ ID NO: 8 (e.g., a T to A substitution) and position 14 in SEQ ID NO: 16 (e.g., T to A substitution).
53. The composition of any one of embodiments 33-52, wherein the chimeric MOMP VD polypeptide comprises a sequence according to any one of SEQ ID NO: 490-505 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto, e.g., wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto.
54. The composition of any one of embodiments 33-36 or 38-53, wherein the composition comprises the nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP VD polypeptide and wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 567-630 or a sequence that has at least 50% (e.g., at least 75%) identity thereto, e.g., wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto.
55. The composition of any one of embodiments 33-36 or 38-54, wherein the composition comprises one or more nucleic acids of (ii)(a)-(d) and wherein the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide and the *Chlamydia sp.* CT812 polypeptide comprise a secretion signal peptide sequence.
56. The composition of embodiment 55, wherein the secretion signal peptide sequence is a viral secretion signal peptide sequence, optionally selected from the group consisting of: an influenza hemagglutinin (HA) secretion signal peptide sequence, a SARS CoV-2 spike secretion signal peptide sequence, a VZV gB secretion signal peptide sequence, a VZV gE secretion signal peptide sequence, a VZV gI secretion signal peptide sequence, a VZV gK secretion signal peptide sequence, a measles F-protein secretion signal peptide sequence, a rubella E1 protein secretion signal peptide sequence, a rubella E2 protein secretion signal peptide sequence, a mumps F-protein secretion signal peptide sequence, an Ebola GP protein secretion signal peptide sequence, and a smallpox 6kDa IC protein secretion signal peptide sequence, optionally wherein the secretion signal peptide sequence comprises an amino acid sequence according to one of the SEQ ID NOs in Table 2 or Table 2.1.

57. The composition of embodiment 56, wherein the secretion signal peptide sequence comprises a secretion signal peptide sequence of HA protein of influenza A virus, *e.g.*, wherein the secretion signal peptide sequence comprises a sequence according to SEQ ID NO: 187 or SEQ ID NO: 188.
58. The composition of any one of embodiments 55-57, wherein one or more of the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide or the *Chlamydia sp.* CT812 polypeptide comprises a heterologous transmembrane domain.
59. The composition of embodiment 58, wherein the transmembrane domain sequence is selected from the group consisting of: an influenza hemagglutinin (HA) transmembrane domain sequence, a SARS CoV-2 spike transmembrane domain sequence, a VZV gB transmembrane domain sequence, a VZV gE transmembrane domain sequence, a VZV gI transmembrane domain sequence, a VZV gK transmembrane domain sequence, a measles F-protein transmembrane domain sequence, a rubella E1 protein transmembrane domain sequence, a rubella E2 protein transmembrane domain sequence, a mumps F-protein transmembrane domain sequence and an Ebola GP protein transmembrane domain sequence, optionally wherein the transmembrane domain comprises an amino acid sequence according to one of the SEQ ID NOs in Table 3.
60. The composition of embodiment 58 or 59, wherein the transmembrane domain comprises the sequence of a transmembrane domain of HA protein of influenza A virus, *e.g.*, wherein the transmembrane domain comprises a sequence according to SEQ ID NO: 813 or SEQ ID NO: 814.
61. The composition of any one of embodiments 33-60, wherein one or more of the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide or the *Chlamydia sp.* CT812 polypeptide comprises a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to a cysteine residue in the respective native *Chlamydia sp.* polypeptide, optionally wherein the single amino acid substitution is a substitution of cysteine with serine.
62. The composition of any one of embodiments 33-61, wherein one or more of the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide or the *Chlamydia sp.* CT812 polypeptide comprises a mutation at one or more (*e.g.*, all) positions corresponding to an N-glycosylation site in the respective native *Chlamydia sp.* polypeptide, optionally wherein the mutation is a single amino acid substitution.
63. The composition of embodiment 62, wherein the *Chlamydia sp.* CT584 polypeptide comprises a single amino acid substitution at a position corresponding to residue 11 of SEQ ID NO: 509 (*e.g.*, a N to Q substitution).
64. The composition of any one of embodiments 33-63, wherein

- (a) the *Chlamydia sp.* CT443 polypeptide comprises a sequence according to SEQ ID NO: 507-508 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto, e.g., wherein the *Chlamydia sp.* CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- 5 (b) the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to any one of SEQ ID NO: 509-512 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto, e.g., wherein the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to any one of SEQ ID NO: 510 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- 10 (c) the *Chlamydia sp.* CT600 polypeptide comprises a sequence according to any one of SEQ ID NO: 513-514 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto; and/or
- (d) the *Chlamydia sp.* CT812 polypeptide comprises a sequence according to any one of SEQ ID NO: 515-535 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto.
- 15
65. The composition of any one of embodiments 33-36 or 38-64, wherein the composition comprises:
- (1) the chimeric MOMP VD polypeptide comprises a sequence according to any one of SEQ ID NO: 490-505 (e.g., SEQ ID NO: 503) or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- 20 (2) the nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP VD polypeptide comprises a nucleotide sequence according to SEQ ID NO: 567-630 (e.g., SEQ ID NO: 617) or a sequence that has at least 50% identity thereto;
- (3) the *Chlamydia sp.* CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- 25 (4) the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 707-710 (e.g., SEQ ID NO: 707) or a sequence that has at least 50% (e.g., at least 75%) identity thereto;
- (5) the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to any one of SEQ ID NO: 509-512 (e.g., SEQ ID NO: 510) or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- 30

(6) the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 711-718 (e.g., SEQ ID NO: 715) or a sequence that has at least 50% (e.g., at least 75%) identity thereto;

(7) the *Chlamydia* sp. CT600 polypeptide comprises a sequence according to any one of SEQ ID NO: 513-514 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto; and/or

(8) the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia* sp. CT600 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 719-722 or a sequence that has at least 50% (e.g., at least 75%) identity thereto;

(9) the *Chlamydia* sp. CT812 polypeptide comprises a sequence according to any one of SEQ ID NO: 515-535 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto; and/or

(10) the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia* sp. CT812 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 723-762 or a sequence that has at least 50% (e.g., at least 75%) identity thereto;

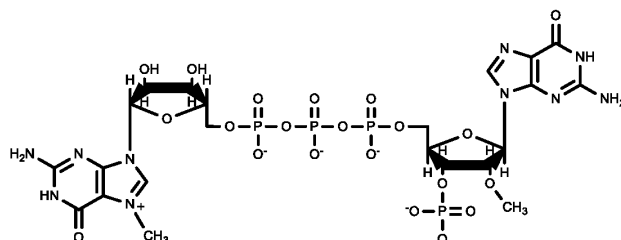
optionally wherein:

the nucleic acids of (ii)(a), wherein the nucleic acid of (ii)(a) comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50%, (at least 75%) identity thereto, and/or

the nucleic acid of (ii)(b), wherein the nucleic acid of (ii)(b) comprises a nucleotide sequence according to SEQ ID NO: 715 or a sequence that has at least 50%, (at least 75%) identity thereto.

66. The composition of any one of embodiments 33-36 or 38-65, wherein nucleic acid of (ii)(a) is a mRNA and the mRNA comprises or consists of (e.g., consists of) the following structural elements:

- a 5' cap, e.g., a 5' cap with the following structure:



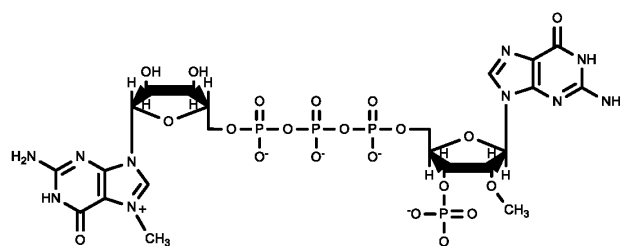
- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;

- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 369;

- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO:839; and
- a polyA tail.

67. The composition of any one of embodiments 33-36 or 38-66, wherein nucleic acid of (ii)(b) is a mRNA and the mRNA comprises or consists of (e.g., consists of) the following structural elements:

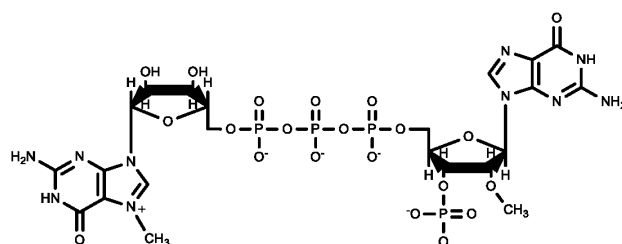
- a 5' cap, e.g., a 5' cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 377;
- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- a polyA tail.

68. The composition of embodiment 54, wherein the nucleic acid comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide is a mRNA and the mRNA comprises or consists of (e.g., consists of) the following structural elements:

- a 5' cap, e.g., a 5' cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 870;

- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and

a polyA tail.

69. The composition of any one of embodiments 33-36 or 38-68, wherein the composition comprises:

- 5 (1) the nucleic acid (e.g., mRNA) of embodiment 16, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of embodiment 18 or 19);

10 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia* sp. MOMP VD polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 68); and

15 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 67); or

- 20 (2) the nucleic acid (e.g., mRNA) of embodiment 16, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of embodiment 18 or 19);

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia* sp. MOMP VD polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 68); and

25 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 715 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 67); or

- 30 (3) the nucleic acid (e.g., mRNA) of embodiment 16, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of embodiment 18 or 19);

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 68);

5 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 67); and

10 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 715 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 67); or

(4) the nucleic acid (e.g., mRNA) of embodiment 15, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

15

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

20 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, wherein the encoding the *Chlamydia sp.* CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

(5) the nucleic acid (e.g., mRNA) of embodiment 15, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

25

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

30

the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, wherein the *Chlamydia sp.* CT584 polypeptide comprises a sequence

according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

- (6) the nucleic acid (e.g., mRNA) of embodiment 15, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, wherein the encoding the *Chlamydia sp.* CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, wherein the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto,

e.g., wherein the composition comprises the nucleic acids as defined in (3) or the nucleic acids as defined in (6).

70. The composition of any one of embodiments 32 or 37-64, wherein the composition comprises:

- (1) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the *Chlamydia sp.* CT443 polypeptide that comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

- (2) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the *Chlamydia sp.* CT584 polypeptide that comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

- (3) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto;

the *Chlamydia sp.* CT443 polypeptide that comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the *Chlamydia sp.* CT584 polypeptide that comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto,

e.g., wherein the composition comprises the polypeptides as defined in (3).

71. A combination comprising a nucleic acid of any one of embodiments 1-19 and:

- (i) a nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*; and/or

- (ii) one or more (e.g., one, two, three or four) of:

(a) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide;

(b) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide;

(c) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide; or

(d) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide.

72. The combination of embodiment 71, wherein the composition comprises

the nucleic acid of (i) and the nucleic acid of (ii)(a);

the nucleic acid of (i) and the nucleic acid of (ii)(b); or

the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b),

e.g., wherein the composition comprises the nucleic acid of (i), the nucleic acid of (ii)(a) and the
5 nucleic acid of (ii)(b),

optionally wherein the nucleic acids are as defined in embodiment 69.

73. A combination comprising a polypeptide of any one of embodiments 20-30 and:

(i) a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric
MOMP VD polypeptide comprises an amino acid sequence comprising two or more
10 *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*; and/or

(ii) one or more (e.g., one, two, three or four) of:

(a) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT443
polypeptide;

(b) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT584
15 polypeptide;

(c) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT600
polypeptide; or

(d) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT812
polypeptide.

20 74. The combination of embodiment 73, wherein the composition comprises

the polypeptide of (i) and the polypeptide of (ii)(a);

the polypeptide of (i) and the polypeptide of (ii)(b); or

the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),

e.g., wherein the composition comprises the polypeptide of (i), the polypeptide of (ii)(a) and the
25 polypeptide of (ii)(b),

optionally wherein the polypeptides are as defined in embodiment 70.

75. The combination of embodiment 71 or 72, wherein the nucleic acids are present in the same
composition or in two or more separate compositions.

76. The combination of embodiment 73 or 74, wherein the polypeptides are present in the same composition or in two or more separate compositions.
77. The composition of embodiment 37, wherein the composition further comprises an adjuvant.
78. The composition of embodiment 36, wherein the LNP comprises at least one cationic lipid, optionally wherein:
- 5 (i) the cationic lipid is selected from the group consisting of OF-02, cKK-E10, OF-Deg-Lin, GL-HEPES-E3-E10-DS-3-E18-1, GL-HEPES-E3-E12-DS-4-E10, GL-HEPES-E3-E12-DS-3-E14, SM-102, ALC-0315, ATX-126, and IM-001 (e.g., GL-HEPES-E3-E12-DS-4-E10 or IM-001); and/or
- 10 (ii) the LNP further comprises a polyethylene glycol (PEG) conjugated (PEGylated) lipid, a cholesterol-based lipid, and a helper lipid, optionally wherein
- the (PEGylated) lipid is DMG-PEG2000 or ALC-0159 (e.g., DMG-PEG2000);
- the cholesterol-based lipid is cholesterol; and/or
- the helper lipid is DOPE or DSPC; and/or
- 15 (iii) the LNP comprises:
- (1) OF-02 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%;
- (2) cKK-E10 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%;
- 20 (3) GL-HEPES-E3-E10-DS-3-E18-1 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%;
- (4) GL-HEPES-E3-E12-DS-4-E10 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%;
- (5) GL-HEPES-E3-E12-DS-3-E14 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%;
- 25 (6) DLin-MC3-DMA (MC3) at a molar ratio of 50%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 38.5%; and DSPC at a molar ratio of 10%; or
- (7) IM-001 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%;

- (8) SM-102 at a molar ratio of 50%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 38.5%, and DSPC at a molar ratio of 10%;
- (9) ALC-0315 at a molar ratio of 46.3%, ALC-0159 at a molar ratio of 1.6%, cholesterol at a molar ratio of 42.7%, and DSPC at a molar ratio of 9.4%;
- 5 (10) ALC-0315 at a molar ratio of 47.4%; ALC-0159 at a molar ratio of 1.7%, cholesterol at a molar ratio of 40.9%; and DSPC at a molar ratio of 10%; or
- (11) ATX-126 a molar ratio of 50%; DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 38.5%, and DSPC at a molar ratio of 10%.
- e.g., wherein the LNP is as defined in (1), (2), (4) or (7), e.g., wherein the LNP is as defined in (4) or (7).
- 10 79. The nucleic acid of any one of embodiments 1-19, the modified MOMP polypeptide of any one of embodiments 20-30, the composition of any one of embodiments 31-70, 77 or 78, or the combination of any one of embodiments 71-76, for use as a medicament.
80. The nucleic acid of any one of embodiments 1-19, the modified MOMP polypeptide of any one of
15 embodiments 20-30, the composition of any one of embodiments 31-70, 77 or 78, or the combination of any one of embodiments 71-76, for use in treating or preventing a *Chlamydia sp.* infection, preferably wherein the infection is a *C. trachomatis* infection.
81. The nucleic acid, the modified MOMP polypeptide, the composition, or the combination, for use according to embodiment 80, wherein the infection is a genital infection.
- 20 82. A vaccine comprising the nucleic acid of any one of embodiments 1-19, the modified MOMP polypeptide of any one of embodiments 20-30, the composition of any one of embodiments 31-70, 77 or 78, or the combination of any one of embodiments 71-76.
83. A nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an
25 amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*.
84. The nucleic acid of embodiment 83, wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.
85. The nucleic acid of embodiment 83 or 84, wherein the chimeric MOMP VD polypeptide comprises conserved domain sequence portions of a native *Chlamydia sp.* MOMP polypeptide flanking each
30 of the two or more MOMP VD sequences.

86. The nucleic acid of any one of embodiments 83-85, wherein the chimeric MOMP VD polypeptide comprises MOMP VD sequences of four different serovars, optionally wherein the different serovars are selected from serovars D, E, F or G of *C. trachomatis*.
87. The nucleic acid of any one of embodiments 83-86, wherein the chimeric MOMP VD polypeptide comprises:
- (i) two MOMP VD1 sequences of different serovars of the *Chlamydia sp.* and/or
 - (ii) two MOMP VD2 sequences of different serovars of the *Chlamydia sp.*; and/or
 - (iii) one MOMP VD3 sequence; and/or
 - (iv) two MOMP VD4 sequences of different serovars of the *Chlamydia sp.*,
- optionally wherein the different serovars are selected from serovars D, E, F, or G of *C. trachomatis*.
88. The nucleic acid of any one of embodiments 83-87, wherein the chimeric MOMP VD polypeptide comprises one MOMP VD sequence of serovar D or E of *C. trachomatis* and one MOMP VD sequence of serovar F or G of *C. trachomatis*.
89. The nucleic acid of embodiment 87 or 88, wherein the chimeric MOMP VD polypeptide comprises at least one (e.g., four) of (i)-(iv):
- (i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, or a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F or G (e.g., a MOMP VD1 sequence of serovar E and a MOMP VD1 sequence of serovar G); and/or
 - (ii) a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, or a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G (e.g., a MOMP VD2 sequence of serovar D and a MOMP VD2 sequence of serovar G); and/or
 - (iii) a MOMP VD3 sequence from serovar G or a VD3 sequence from serovar F (e.g., serovar F); and/or
 - (iv) a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G, or a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F (e.g., a MOMP VD4 sequence of serovar D and a MOMP VD4 sequence of serovar F),
- wherein the serovars D, E, F or G are of *C. trachomatis*.
90. The nucleic acid of embodiment 89, wherein the chimeric MOMP VD polypeptide comprises:
- (i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, a

MOMP VD3 sequence from serovar G, a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G; or

- (ii) a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G, a MOMP VD3 sequence from serovar F, a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F,

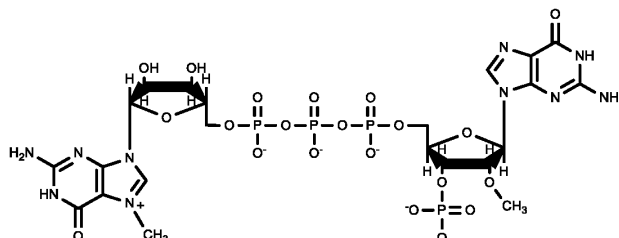
wherein the serovars D, E, F or G are of *C. trachomatis*.

91. The nucleic acid of embodiment 90, wherein the chimeric MOMP VD polypeptide comprises MOMP VD sequences according to (ii).
92. The nucleic acid of any one of embodiments 85-91, wherein the conserved domain sequence portions are portions of conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide flanking the VD in its native *Chlamydia sp.* MOMP polypeptide.
93. The nucleic acid of any one of embodiments 85-92, wherein each conserved domain sequence portion comprises between 3 and 30 amino acid residues of a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, wherein the between 3 and 30 amino acid residues are immediately adjacent to the VD sequence in its native *Chlamydia sp.* MOMP polypeptide.
94. The nucleic acid of any one of embodiments 83-93, wherein the chimeric *Chlamydia sp.* MOMP VD polypeptide comprises a secretion signal peptide sequence.
95. The nucleic acid of embodiment 94, wherein the secretion signal peptide sequence is a viral secretion signal peptide sequence, optionally selected from the group consisting of: an influenza hemagglutinin (HA) secretion signal peptide sequence, a SARS CoV-2 spike secretion signal peptide sequence, a VZV gB secretion signal peptide sequence, a VZV gE secretion signal peptide sequence, a VZV gI secretion signal peptide sequence, a VZV gK secretion signal peptide sequence, a measles F-protein secretion signal peptide sequence, a rubella E1 protein secretion signal peptide sequence, a rubella E2 protein secretion signal peptide sequence, a mumps F-protein secretion signal peptide sequence, an Ebola GP protein secretion signal peptide sequence, and a smallpox 6kDa IC protein secretion signal peptide sequence, optionally wherein the secretion signal peptide sequence comprises an amino acid sequence according to any one of the SEQ ID NOs in Table 2 or Table 2.1.
96. The nucleic acid of embodiment 95, wherein the secretion signal peptide sequence comprises a secretion signal peptide sequence of HA protein of influenza A virus, *e.g.*, wherein the secretion signal peptide sequence comprises a sequence according to SEQ ID NO: 187 or SEQ ID NO: 188.

97. The nucleic acid of any one of embodiments 83-96, wherein the chimeric MOMP VD polypeptide comprises a single amino acid substitution at one or more (*e.g.*, all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the single amino acid substitution is a substitution of cysteine with serine.
- 5 98. The nucleic acid of any one of embodiments 83-97, wherein the chimeric MOMP VD polypeptide comprises a mutation at one or more (*e.g.*, all) positions corresponding to a glycosylation site, optionally an N-glycosylation site, in a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the mutation is a single amino acid substitution.
- 10 99. The nucleic acid of embodiment 98, wherein chimeric MOMP VD polypeptide comprises a single amino acid substitution at each of the amino acid residues corresponding to position 9 of SEQ ID NO: 9 (*e.g.*, N to A substitution), position 11 of SEQ ID NO: 17 (*e.g.*, T to A substitution), position 17 of SEQ ID NO: 6 (*e.g.*, S to A substitution), positions 4 and 21 of SEQ ID NO: 18 (*e.g.*, N to A and S to A substitutions, respectively), position 14 of SEQ ID NO: 8 (*e.g.*, a T to A substitution) and position 14 in SEQ ID NO: 16 (*e.g.*, T to A substitution)
- 15 100. The nucleic acid of any one of embodiments 83-99, wherein the chimeric MOMP VD polypeptide comprises a sequence according to any one of SEQ ID NO: 490-505 or a sequence that has at least 70% (*e.g.*, at least 90 or 95%) identity thereto, *e.g.*, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 70% (*e.g.*, at least 90 or 95%) identity thereto.
- 20 101. The nucleic acid of any one of embodiments 83-100, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 567-630 or a sequence that has at least 50% (*e.g.*, at least 75%) identity thereto, *e.g.*, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (*e.g.*, at least 75%) identity thereto.
- 25 102. The nucleic acid of any one of embodiments 83-101, wherein the nucleic acid is a messenger RNA (mRNA), optionally wherein
- (i) the mRNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (poly(A)) sequence;
- (ii) the mRNA is unmodified or comprises at least one chemical modification, optionally
- 30 wherein the mRNA comprises at least one chemical modification, *e.g.*, wherein the chemical modification comprises N1-methylpseudouridine; and/or
- (iii) the mRNA is a self-replicating mRNA or a non-replicating mRNA, *e.g.*, a non-replicating mRNA.

103. The nucleic acid of embodiment 102, wherein the mRNA comprises or consists of (e.g., consists of) the following structural elements:

- a 5' cap, e.g., a 5' cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 870;
- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- a polyA tail.

104. The nucleic acid of embodiment 103, wherein the mRNA is chemically modified and wherein the chemical modification comprises or consists of (e.g., consists of) N1-methylpseudouridine in place of every uridine.

105. A chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*

106. The chimeric MOMP VD polypeptide of embodiment 105, wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.

107. The chimeric MOMP VD polypeptide of embodiments 105 or 106, wherein the chimeric MOMP VD polypeptide comprises conserved domain sequence portions of a native *Chlamydia sp.* MOMP polypeptide flanking each of the two or more MOMP VD sequences.

108. The chimeric MOMP VD polypeptide of any one of embodiments 105-107, wherein the chimeric MOMP VD polypeptide comprises MOMP VD sequences of four different serovars, optionally wherein the different serovars are selected from serovars D, E, F or G of *C. trachomatis*.

109. The chimeric MOMP VD polypeptide of any one of embodiments 105-108, wherein the chimeric MOMP VD polypeptide comprises:

- (i) two MOMP VD1 sequences of different serovars of the *Chlamydia sp.* and/or

- (ii) two MOMP VD2 sequences of different serovars of the *Chlamydia sp.*; and/or
 - (iii) one MOMP VD3 sequence; and/or
 - (iv) two MOMP VD4 sequences of different serovars of the *Chlamydia sp.*,
- optionally wherein the different serovars are selected from serovars D, E, F or G of *C. trachomatis*.

5 110. The chimeric MOMP VD polypeptide of any one of embodiments 105-109, wherein the chimeric MOMP VD polypeptide comprises one MOMP VD sequence of serovar D or E of *C. trachomatis* and one MOMP VD sequence of serovar F or G of *C. trachomatis*.

111. The chimeric MOMP VD polypeptide of embodiment 109 or 110, wherein the chimeric MOMP VD polypeptide comprises at least one (e.g., four) of (i)-(iv):

10 (i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, or a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F or G (e.g., a MOMP VD1 sequence of serovar E and a MOMP VD1 sequence of serovar G); and/or

(ii) a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, or a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G (e.g., a MOMP
15 VD2 sequence of serovar D and a MOMP VD2 sequence of serovar G); and/or

(iii) a MOMP VD3 sequence from serovar G or a VD3 sequence from serovar F (e.g., serovar F); and/or

(iv) a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G, or a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F (e.g., a
20 MOMP VD4 sequence of serovar D and a MOMP VD4 sequence of serovar F),

wherein the serovars D, E, F or G are of *C. trachomatis*.

112. The chimeric MOMP VD polypeptide of embodiment 111, wherein the chimeric MOMP VD polypeptide comprises:

25 (i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, a MOMP VD3 sequence from serovar G, a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G; or

(ii) a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G, a
30 MOMP VD3 sequence from serovar F, a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F,

wherein the serovars D, E, F or G are of *C. trachomatis*.

113. The chimeric MOMP VD polypeptide of embodiment 112, wherein the chimeric MOMP VD polypeptide comprises MOMP VD sequences according to (ii).
114. The chimeric MOMP VD polypeptide of any one of embodiments 107-113, wherein the conserved domain sequence portions are portions of conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide flanking the VD in its native *Chlamydia sp.* MOMP polypeptide.
115. The chimeric MOMP VD polypeptide of any one of embodiments 107-114, wherein each conserved domain sequence portion comprises between 3 and 30 amino acid residues of a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, wherein the between 3 and 30 amino acid residues are immediately adjacent to the VD sequence in its native *Chlamydia sp.* MOMP polypeptide.
116. The chimeric MOMP VD polypeptide of any one of embodiments 105-115, wherein the chimeric MOMP VD polypeptide comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the single amino acid substitution is a substitution of cysteine with serine.
117. The chimeric MOMP VD polypeptide of any one of embodiments 105-116, wherein the chimeric MOMP VD polypeptide comprises a mutation at one or more (e.g., all) positions corresponding to a glycosylation site, optionally an N-glycosylation site, in a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the mutation is a single amino acid substitution.
118. The chimeric MOMP VD polypeptide of embodiment 117, wherein chimeric MOMP VD polypeptide comprises a single amino acid substitution at each of the amino acid residues corresponding to position 9 of SEQ ID NO: 9 (e.g., N to A substitution), position 11 of SEQ ID NO: 17 (e.g., T to A substitution), position 17 of SEQ ID NO: 6 (e.g., S to A substitution), positions 4 and 21 of SEQ ID NO: 18 (e.g., N to A and S to A substitutions, respectively), position 14 of SEQ ID NO: 8 (e.g., a T to A substitution) and position 14 in SEQ ID NO: 16 (e.g., T to A substitution)
119. The chimeric MOMP VD polypeptide of any one of embodiments 105-118, wherein the chimeric MOMP VD polypeptide comprises a sequence according to any one of SEQ ID NO: 490-505 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto, e.g., wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto.

120. A composition comprising the nucleic acid of any one of embodiments 83-104, preferably wherein the composition is an immunogenic composition.
121. A composition comprising the polypeptide of any one of embodiments 105-119, preferably wherein the composition is an immunogenic composition.
- 5 122. The composition of embodiment 120, wherein the composition further comprises:
- (i) a nucleic acid that comprises a nucleotide sequence encoding a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences;
10 and/or
- (ii) one or more (e.g., one, two, three or four) of:
- (a) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide;
- (b) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide;
15
- (c) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide; or
- (d) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide.
- 20 123. The composition of embodiment 122, wherein the composition comprises the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b).
124. The composition of embodiment 122 or 123, wherein one or more nucleic acids is a mRNA, optionally wherein
- (i) the mRNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (poly(A)) sequence;
25
- (ii) the mRNA is unmodified or comprises at least one chemical modification, optionally wherein the mRNA comprises at least one chemical modification e.g., wherein the chemical modification comprises N1-methylpseudouridine; and/or
- (iii) the mRNA is a self-replicating mRNA or a non-replicating mRNA, e.g., a non-replicating
30 mRNA.

125. The composition of any one of embodiments 120 or 122-124, wherein the composition further comprises a lipid nanoparticle (LNP), optionally wherein the nucleic acid is encapsulated in the LNP.
126. The composition of any one of embodiments 122-125, wherein the nucleic acid encoding the modified MOMP polypeptide is as defined in any one of embodiments 1-19.
127. The composition of any one of embodiments 122-126, wherein any one of the nucleic acid that comprises a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, the nucleic acid that comprises a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, the nucleic acid that comprises a nucleotide sequence encoding the *Chlamydia sp.* CT600 polypeptide or the nucleic acid that comprises a nucleotide sequence encoding the *Chlamydia sp.* CT812 polypeptide is as defined in any one of embodiments 55-68.
128. The composition of any one of embodiments 122-127, wherein the composition comprises:
- (1) the nucleic acid (e.g., mRNA) of embodiment 16, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of embodiment 18 or 19);
- the nucleic acid (e.g., mRNA) of embodiment 101, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 68); and
- the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 67); or
- (2) the nucleic acid (e.g., mRNA) of embodiment 16, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of embodiment 18 or 19);
- the nucleic acid (e.g., mRNA) of embodiment 101, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 68); and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 715 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 67); or

- 5 (3) the nucleic acid (e.g., mRNA) of embodiment 16, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of embodiment 18 or 19);

10 the nucleic acid (e.g., mRNA) of embodiment 101, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 68);

15 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 67); and

20 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 715 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of embodiment 67); or

- (4) the nucleic acid (e.g., mRNA) of embodiment 15, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

25 the nucleic acid (e.g., mRNA) of embodiment 101, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

30 the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the encoding the *Chlamydia* sp. CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

- (5) the nucleic acid (e.g., mRNA) of embodiment 15, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) of embodiment 101, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

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the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, wherein the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

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- (6) the nucleic acid (e.g., mRNA) of embodiment 15, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) of embodiment 101, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto;

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the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, wherein the encoding the *Chlamydia sp.* CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

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the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, wherein the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto,

e.g., wherein the composition comprises the nucleic acids as defined in (3) or the nucleic acids as defined in (6).

129. The composition of embodiment 121, wherein the composition further comprises:

25

- (i) a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences; and/or

- (ii) one or more (e.g., one, two, three, or four) of:

30

- (a) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT443 polypeptide;

- (b) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT584 polypeptide;
- (c) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT600 polypeptide; or
- 5 (d) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT812 polypeptide.
130. The composition of embodiment 129, wherein the composition comprises
- the polypeptide of (i) and the polypeptide of (ii)(a); or
- the polypeptide of (i) and the polypeptide of (ii)(b); or
- 10 the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),
- e.g., wherein the composition comprises the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b).
131. The composition of any one of embodiments 129 or 130, wherein the modified MOMP polypeptide is as defined in any one of embodiments 20-30.
- 15 132. The composition of any one of embodiments 129-131, wherein any one of the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide or the *Chlamydia sp.* CT812 polypeptide is as defined in any one of embodiments 55-68.
133. The composition of any one of embodiments 129-132, wherein the composition comprises:
- (1) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a
- 20 sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and
- the *Chlamydia sp.* CT443 polypeptide that comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or
- 25 (2) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

- the *Chlamydia sp.* CT584 polypeptide that comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or
- (3) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- 5 the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto;
- the *Chlamydia sp.* CT443 polypeptide that comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and
- the *Chlamydia sp.* CT584 polypeptide that comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto,
- 10 e.g., wherein the composition comprises the polypeptides as defined in (3).
134. The composition of any one of embodiments 121 or 129-133, wherein the composition further comprises an adjuvant.
135. The composition of embodiment 125, wherein the LNP comprises at least one cationic lipid,
- 15 optionally wherein:
- (i) the cationic lipid is selected from the group consisting of OF-02, cKK-E10, OF-Deg-Lin, GL-HEPES-E3-E10-DS-3-E18-1, GL-HEPES-E3-E12-DS-4-E10, GL-HEPES-E3-E12-DS-3-E14, SM-102, ALC-0315, ATX-126, and IM-001 (e.g., GL-HEPES-E3-E12-DS-4-E10 or IM-001); and/or
- 20 (ii) the LNP further comprises a polyethylene glycol (PEG) conjugated (PEGylated) lipid, a cholesterol-based lipid, and a helper lipid, optionally wherein
- the (PEGylated) lipid is DMG-PEG2000 or ALC-0159 (e.g., DMG-PEG2000);
- the cholesterol-based lipid is cholesterol; and/or
- the helper lipid is DOPE or DSPC; and/or
- 25 (iii) the LNP comprises:
- (1) OF-02 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
- (2) cKK-E10 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;

- (3) GL-HEPES-E3-E10-DS-3-E18-1 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
- (4) GL-HEPES-E3-E12-DS-4-E10 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
- 5 (5) GL-HEPES-E3-E12-DS-3-E14 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
- (6) DLin-MC3-DMA (MC3) at a molar ratio of 50%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 38.5%, and DSPC at a molar ratio of 10%; or
- 10 (7) IM-001 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%,
- (8) SM-102 at a molar ratio of 50%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 38.5%, and DSPC at a molar ratio of 10%;
- (9) ALC-0315 at a molar ratio of 46.3%, ALC-0159 at a molar ratio of 1.6%, cholesterol at a molar ratio of 42.7%, and DSPC at a molar ratio of 9.4%;
- 15 (10) ALC-0315 at a molar ratio of 47.4%, ALC-0159 at a molar ratio of 1.7%, cholesterol at a molar ratio of 40.9%, and DSPC at a molar ratio of 10%; or
- (11) ATX-126 at a molar ratio of 50%; DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 38.5%, and DSPC at a molar ratio of 10%,
- e.g., wherein the LNP is as defined in (1), (2), (4) or (7), e.g., wherein the LNP is as defined in (4) or (7).
- 20
136. The composition of any one of embodiments 120-135, wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.
137. A combination comprising a nucleic acid of any one of embodiments 83-104 and:
- 25 (i) a nucleic acid that comprises a nucleotide sequence encoding a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences; and/or
- (ii) one or more (e.g., one, two, three, or four) of:

- (a) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide;
- (b) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide;
- 5 (c) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide; or
- (d) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide.
138. The combination of embodiment 137, wherein the composition comprises
- 10 the nucleic acid of (i) and the nucleic acid of (ii)(a);
- the nucleic acid of (i) and the nucleic acid of (ii)(b); or
- the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b),
- e.g., wherein the composition comprises the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b),
- 15 optionally wherein the nucleic acids are as defined in embodiment 128.
139. A combination comprising a polypeptide of any one of embodiments 105-119 and:
- (i) a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain
- 20 sequences; and/or
- (ii) one or more (e.g., one, two, three, or four) of:
- (a) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT443 polypeptide;
- (b) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT584
- 25 polypeptide;
- (c) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT600 polypeptide; or
- (d) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT812 polypeptide.

140. The combination of embodiment 139, wherein the composition comprises
the polypeptide of (i) and the polypeptide of (ii)(a);
the polypeptide of (i) and the polypeptide of (ii)(b); or
the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),
5 e.g., wherein the composition comprises the polypeptide of (i), the polypeptide of (ii)(a) and the
polypeptide of (ii)(b),
optionally wherein the polypeptides are as defined in embodiment 133.
141. The combination of embodiment 137 or 138, wherein the nucleic acids are present in the same
composition or in two or more separate compositions.
- 10 142. The combination of embodiment 139 or 140, wherein the polypeptides are present in the same
composition or in two or more separate compositions.
143. The nucleic acid of any one of embodiments 83-104, the chimeric MOMP VD polypeptide of any
one of embodiments 105-119, the composition of any one of embodiments 120-136, or the
combination of any one of embodiments 136-142, for use as a medicament.
- 15 144. The nucleic acid of any one of embodiments 83-104, the chimeric MOMP VD polypeptide of any
one of embodiments 105-119, the composition of any one of embodiments 120-136, or the
combination of any one of embodiments 136-142, for use in treating or preventing a *Chlamydia sp.*
infection, preferably wherein the infection is a *C. trachomatis* infection.
- 20 145. The nucleic acid, the chimeric MOMP VD polypeptide, the composition, or the combination, for
use according to embodiment 144, wherein the infection is a genital infection.
146. A vaccine comprising the nucleic acid of any one of embodiments 83-104, the chimeric MOMP
VD polypeptide of any one of embodiments 105-119, the composition of any one of embodiments
120-136, or the combination of any one of embodiments 136-142.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows induction of cross-serovar-responsive IFN γ -producing T cells in mice immunised with native SS-MOMP mRNA delivering wild type (WT) full-length MOMP (“mRNA MNR MOMP E FL”) or MOMP without variable domains VD2 and VD4 (“mRNA UNR MOMP E DII:IV”), in response to stimulation with pools of overlapping peptides of MOMP serovar D, E or F. MNR = modified mRNA (in which all uridine nucleosides were replaced with 1-methyl pseudo uridine). UNR = unmodified mRNA (containing unmodified uridine nucleosides).

FIGURE 2 shows induction of cross-serovar-responsive (A) CD4+IFN γ + T cells and (B) CD8+IFN γ + T cells in mice immunised with “mRNA MNR MOMP E FL” or “mRNA UNR MOMP E DII:IV”, in response to stimulation with pools of overlapping peptides of MOMP serovar E or F.

FIGURE 3 shows reciprocal dilution IgG titres in mice immunised with LNP-formulated mRNAs encoding recombinant serovar E MOMP, assessed using plate ELISA.

FIGURE 4 shows T cell responses in mice immunised with LNP-formulated mRNAs encoding recombinant serovar E MOMP in response to stimulation with (A) UV inactivated EBs, (B) “rMOMP” (recombinant serovar E MOMP protein), and (C) a pool of overlapping serovar E MOMP peptides, assessed in splenic IFN γ ELISPOT.

FIGURE 5A and **5B** show the level of IFN γ production by T cells in mice following immunisation with MOMP mRNA constructs in which one or more variable domain (VD) loops are removed, in response to stimulation with recall antigen (pools of overlapping peptides of serovar E MOMP).

FIGURE 6 shows the induction in mice of (A) IFN γ -producing T cells (in response to MOMP serovar E peptide pools), (B) CD4+ IFN γ T cells, and (C) CD8+ IFN γ T cells following immunisation with “mRNA UNR MOMP E DII:IV” or recombinant serovar E MOMP protein.

FIGURE 7 shows the induction of cross-serovar-responsive IFN γ -producing T cells in mice following immunisation with mRNAs encoding modified MOMP polypeptide antigens in which VD loops have been removed (MOMP_P3_ssHA1 (“P3”), MOMP_P5_ssHA1_C2S (“P5 C2S”)), or full-length MOMP constructs MOMP_serE_FL_ssHA1 (“FL”), MOMP_serE_FL_ssHA1_C2S_Glycneg (“FL C2S Glycneg”). Recall antigens were overlapping peptides of MOMP serovar D, E, F, G or J.

FIGURE 8 shows induction of cross-serovar-responsive IL-5-producing T cells in mice following immunisation with MOMP_P3_ssHA1 (“P3”), MOMP_P5_ssHA1_C2S (“P5 C2S”), MOMP_serE_FL_ssHA1 (“FL”), or MOMP_serE_FL_ssHA1_C2S_Glycneg (“FL C2S Glycneg”). Recall antigens were overlapping peptides of MOMP serovar D, E, F, G or J.

FIGURE 9 shows the induction of cross-serovar-responsive IFN γ -producing T cells in mice following immunisation with mRNAs encoding modified MOMP polypeptide antigens in which VD loops have been removed (MOMP_P3_ssHA1 (“P3”), MOMP_P3_ssHA1_C2S (“P3 C2S”), MOMP_P5_ssHA1 (“P5”), MOMP_P5_ssHA1_C2S (“P5 C2S”), or full-length MOMP constructs MOMP_serE_FL_ssHA1 (“SerE FL”), MOMP_serD_FL_ssHA1 (“SerD FL”) according to the study detailed in 7.2. Recall antigens were overlapping peptides of (A) MOMP serovar E or (B) MOMP serovar D, F, G or J.

FIGURE 10 shows induction in mice of (A) CD4+ IFN γ T cell responses and (B) CD8+ IFN γ T cell responses following immunisation with MOMP_P3_ssHA1 (“P3”), MOMP_P5_ssHA1_C2S (“P5 C2S”), MOMP_serE_FL_ssHA1 (“FL”), or MOMP_serE_FL_ssHA1_C2S_Glycneg (“FL C2S Glycneg”).

FIGURE 11 shows induction in mice of (A) CD4+ IFN γ T cell responses and (B) CD8+ IFN γ T cell responses following immunisation with MOMP_P3_ssHA1 (“P3”), MOMP_P3_ssHA1_C2S (“P3 C2S”), MOMP_P5_ssHA1 (“P5”), MOMP_P5_ssHA1_C2S (“P5 C2S”), or full-length MOMP constructs MOMP_serE_FL_ssHA1 (“SerE FL”), MOMP_serD_FL_ssHA1 (“SerD FL”) according to the study detailed in 7.2.

FIGURE 12 shows the percentage of CD4+ T cells in mice that produce IFN γ (“IFNg”), IL2 and/or TNF α (“TNFa”) (singularly and in the various combinations thereof), after mice are immunised with MOMP_P3_ssHA1 (“P3”), MOMP_P5_ssHA1_C2S (“P5 C2S”), MOMP_serE_FL_ssHA1 (“FL”), or MOMP_serE_FL_ssHA1_C2S_Glycneg (“FL C2S Glycneg”).

FIGURE 13 shows the percentage of CD4+ T cells in mice that produce IFN γ (“IFNg”), IL2 and/or TNF α (“TNFa”) (singularly and in the various combinations thereof), after mice are immunised with MOMP_P3_ssHA1 (“P3”), MOMP_P3_ssHA1_C2S (“P3 C2S”), MOMP_P5_ssHA1 (“P5”), MOMP_P5_ssHA1_C2S (“P5 C2S”), or full-length MOMP constructs MOMP_serE_FL_ssHA1 (“SerE FL”), MOMP_serD_FL_ssHA1 (“SerD FL”) according to the study detailed in 7.2.

FIGURE 14 shows levels of IgG binding to recombinant MOMP (rMOMP) protein, as measured using ELISA, following immunisation of mice with MOMP_P3_ssHA1 (“P3”), MOMP_P3_ssHA1_C2S (“P3 C2S”), MOMP_P5_ssHA1 (“P5”), MOMP_P5_ssHA1_C2S (“P5 C2S”), or full-length MOMP constructs MOMP_serE_FL_ssHA1 (“FL”), MOMP_serD_FL_ssHA1 (“SerD FL”) according to the study detailed in 7.2.

FIGURE 15 shows IFN γ T cell responses in mice following immunisation with recombinant (r) non-MOMP polypeptides “rCT584”, “rCT812”, “rCT600” or “rCT443”, recombinant MOMP protein (“rMOMP”) or mRNA encoding MOMP serovar E lacking VD loops 1-4 (“mRNA MOMP Δ VD1234”). Pools of overlapping peptides of the respective native MOMP or non-MOMP were used as recall antigens.

FIGURE 16 shows IFN γ T cell responses in mice following immunisation with non-MOMP fragment mRNA constructs (CT812: 1-1530, CT812: 1-761, Nan96-SS:CT812: 32-761, CT600: 1-188, CT443: 1-576, Nan96-SS:CT443: 32-576), or with mRNA encoding MOMP serovar E lacking VD loops 1-4 ("MOMP Δ VD1234"). Pools of overlapping peptides of the respective native MOMP or non-MOMP were used as recall antigens.

FIGURE 17 shows IFN γ T cell responses in mice following immunisation with mRNA encoding non-MOMP *Ct* antigens "CT443" (Nan96-SS:CT443: 32-576), "CT584" (Nan96HA-SS:CT584:2-183), "CT600" (Nan96HA-SS:CT600:2-188) or "CT812" (Nan96-SS:CT812:32-761), or with recombinant MOMP serovar E protein, in response to stimulation with respective overlapping peptide pools. Responses are shown for splenocytes collected at days eleven (D11) and fourteen (D14) post-boost.

FIGURE 18 shows induction in mice of (A) CD4⁺ IFN γ T cell responses and (B) CD8⁺ IFN γ T cell responses following immunisation with mRNA encoding non-MOMP *Ct* antigens "CT443" (Nan96-SS:CT443: 32-576), "CT584" (Nan96HA-SS:CT584:2-183), "CT600" (Nan96HA-SS:CT600:2-188) or "CT812" (Nan96-SS:CT812:32-761), or with recombinant MOMP serovar E protein.

FIGURE 19 shows antigen-specific IgG levels, as measured using ELISA, following immunisation with mRNA encoding non-MOMP *Ct* antigens CT443_ssHA1 ("CT443"), CT443_ssHA1_GlycNeg ("CT443_GN"), CT584_ssHA1 ("CT584"), CT584_ssHA1_GlycNeg ("CT584_GN"), CT584_ssHA1_C2S ("CT584_C2S"), CT584_ssHA1_GlycNeg_C2S ("CT584_GN_C2S"), CT600_trunc_ssHA1 ("CT600_trunc"), CT600_trunc_ssHA1_GlycNeg ("CT600_trunc_GN"), CT812_pass-domain_ssHA1 ("CT812_pass-dom"), CT812_pass-domain_ssHA1_C2S ("CT812_pass-dom_C2S"), CT812_ext-pass-domain_ssHA1 ("CT812_ext-pass-dom"), CT812_ext-pass-domain_ssHA1_C2S ("CT812_ext-pass-dom_C2S").

FIGURE 20 shows induction of IFN γ -producing T cells in mice following immunisation with mRNA encoding non-MOMP *Ct* antigens CT443_ssHA1 ("CT443"), CT584_ssHA1_GlycNeg ("CT584_GlycNeg"), or CT600_trunc_ssHA1_GlycNeg ("CT600_GlycNeg"), or with mRNA encoding recombinant MOMP serovar E protein as a control.

FIGURE 21 shows induction in mice of (A) CD4⁺ IFN γ T cell responses and (B) CD8⁺ IFN γ T cell responses, following immunisation with mRNA encoding non-MOMP *Ct* antigens CT443_ssHA1 ("CT443"), CT584_ssHA1_GlycNeg ("CT584_GlycNeg"), or CT600_trunc_ssHA1_GlycNeg ("CT600_GlycNeg"), or with mRNA encoding recombinant MOMP serovar E protein as a control.

FIGURE 22 shows the percentage of (A) CD4⁺ T cells and (B) CD8⁺ T cells in mice producing each of IFN γ ("IFN γ "), IL2, and/or TNF α ("TNF α ") (singularly and in the various combinations thereof), after mice are immunised with mRNA encoding non-MOMP *Ct* antigens CT443_ssHA1 ("CT443"),

CT584_ssHA1_GlycNeg (“CT584_GlycNeg”), or CT600_trunc_ssHA1_GlycNeg (“CT600_GlycNeg”), or with mRNA encoding recombinant MOMP serovar E protein as a control.

FIGURE 23 shows antigen-specific IgG levels, as measured using ELISA, following immunisation of mice with mRNA encoding non-MOMP *Ct* antigens CT443_ssHA1 (“CT443 mRNA”) CT584_ssHA1_GlycNeg (“CT584 mRNA”), CT600_trunc_ssHA1_GlycNeg (“CT600 mRNA”) or with one of recombinant proteins (rProt) CT584, CT600 and CT443.

FIGURE 24 shows the level of IgG binding to EBs of serovar E, as measured using ELISA, following immunisation of mice with mRNA encoding non-MOMP *Ct* antigens CT443_ssHA1 (“CT443”), CT443_ssHA1_GlycNeg (“CT443_GN”), CT584_ssHA1 (“CT584”), CT584_ssHA1_GlycNeg (“CT584_GN”), CT584_ssHA1_C2S (“CT584_C2S”), CT600_trunc_ssHA1_GlycNeg (“CT600_trunc”), CT600_trunc_ssHA1_GlycNeg (“CT600_trunc_GN”), CT812_pass-domain_ssHA1 (“CT812_pass-dom”), CT812_pass-domain_ssHA1_C2S (“CT812_pass-dom_CS2”), CT812_ext-pass-domain_ssHA1 (“CT812_ext-pass-dom”), or CT812_ext-pass-domain_ssHA1_C2S (“CT812_ext-pass-dom_C2S”).

FIGURE 25 shows the level of IgG binding to EBs of serovar D, as measured using ELISA, following immunisation of mice with mRNA encoding non-MOMP *Ct* antigens as described above in the legend of Figure 24.

FIGURE 26 shows the level of IgG binding to EBs of serovar G, as measured using ELISA, following immunisation of mice with mRNA encoding non-MOMP *Ct* antigens as described above in the legend of Figure 24.

FIGURE 27 shows antigen-specific IgG levels, as measured using ELISA, following immunisation of mice with mRNA encoding various chimeric MOMP VD polypeptides, with mRNA encoding full-length MOMP polypeptides, or with rMOMP protein.

FIGURE 28 shows the level of IgG binding to EBs of serovar E, as measured using ELISA, following immunisation of mice with mRNA encoding various chimeric MOMP VD polypeptides, with mRNA encoding full-length MOMP polypeptides, or with rMOMP protein.

FIGURE 29 shows the level of IgG binding to EBs of serovar D, as measured using ELISA, following immunisation of mice with mRNA encoding various chimeric MOMP VD polypeptides, with mRNA encoding full-length MOMP polypeptides, or with rMOMP protein.

FIGURE 30 shows the level of IgG binding to EBs of serovar G, as measured using ELISA, following immunisation of mice with mRNA encoding various chimeric MOMP VD polypeptides, with mRNA encoding full-length MOMP polypeptides, or with rMOMP protein.

FIGURE 31 shows induction in mice of the (A) CD4+ IFN γ T cell response, (B) CD8+ IFN γ T cell response, and (C) CD4+IFN γ +IL2+TNF α + cell population, following immunisation with mRNA encoding MOMP_P3_ssHA1 (“P3”) in two different LNP formulations or MOMP_VDcomb2-extS_ssHA1_Glycneg (“C2-S-GN”). Empty LNPs served as controls.

5 **FIGURE 32** shows (A) the level of antigen-specific IgG against recombinant MOMP (rMOMP) of serovar E and (B) the level of IgG binding to EBs of serovar E, as measured using ELISA, following immunisation of mice with mRNA encoding MOMP_VDcomb2-extS_ssHA1_Glycneg (“C2-S-GN”) in two different LNP formulations. Empty LNPs and pre-immune sera (“pre-immun”) served as controls.

10 **FIGURE 33** shows induction in mice of (A) the CD4+ IFN γ T cell response and (B) the percentage of CD4+ T cells in mice that produce IFN γ (“IFN γ ”), IL2 and/or TNF α (singularly and in the various combinations thereof), following immunisation with the multivalent composition as described in Example 12, formulated in two different LNP formulations and at three different doses. Empty LNPs corresponding to the amount of LNP used for the doses of 14.4 μ g and 4.8 μ g mRNA (“equiv. LNP”) served as controls.

15 **FIGURE 34** shows induction in mice of (A) the CD8+ IFN γ T cell response and (B) the percentage of CD8+ T cells in mice that produce IFN γ (“IFN γ ”), IL2 and/or TNF α (singularly and in the various combinations thereof), following immunisation with a multivalent composition as described in Example 12, formulated in two different LNP formulations and at three different doses. Empty LNPs corresponding to the amount of LNP used for the doses of 14.4 μ g and 4.8 μ g mRNA (“equiv. LNP”) served as controls.

20 **FIGURE 35** shows (A) the level of antigen-specific IgG against recombinant MOMP (rMOMP) of serovar E and (B) the level of IgG binding to EBs of serovar E, as measured using ELISA, following immunisation of mice with a multivalent composition as described in Example 12, in two different LNP formulations and at three different doses. IgG induced by empty LNPs corresponding to the amount of LNP used for the doses of 14.4 μ g and 4.8 μ g mRNA (“equiv. LNP”) and in pre-immune sera (“pre-immun”) are shown as controls.

25 **FIGURE 36** shows the level of antigen-specific IgG against the (A) CT443 protein and (B) CT584 protein, as measured using ELISA, following immunisation of mice with a multivalent composition as described in Example 12, in two different LNP formulations and at three different doses. IgG induced by empty LNPs corresponding to the amount of LNP used for the doses of 14.4 μ g and 4.8 μ g mRNA (“equiv. LNP”) and in pre-immune sera (“pre-immun”) are shown as controls.

30 **FIGURE 37** shows levels of IgG binding to EBs of serovar E, as measured using ELISA, following immunisation of mice with mRNA encoding monovalent polypeptides or a multivalent composition as described in Example 13, formulated in either (A) “Lipid D” or (B) “Lipid G”. *** corresponds to a p-value of p<0.001.

FIGURE 38 shows levels of IgG binding to EBs of serovar G, as measured using ELISA, following immunisation of mice with mRNA encoding monovalent polypeptides or a multivalent composition as described in Example 13, formulated in either (A) “Lipid D” or (B) “Lipid G”. *** corresponds to a p-value of $p < 0.001$.

- 5 **FIGURE 39** shows levels of IgG binding to recombinant proteins (A and B: MOMP; C and D: CT443; E and F: CT584), as measured using ELISA, following immunisation of mice with mRNA encoding monovalent polypeptides or a multivalent composition as described in Example 13, formulated in either (A, C, E) “Lipid D” or (B, D, F) “Lipid G”.

MODES FOR CARRYING OUT THE INVENTION

10 **EXAMPLE 1 – Design of *C. trachomatis* (Ct) antigen constructs**

Design of T cell constructs (modified MOMP polypeptide constructs)

mRNA constructs encoding modified MOMP polypeptide antigens were prepared with the aim of eliciting MOMP-specific T cell responses, particularly MOMP specific IFN γ + CD4+ T cells – i.e. MOMP T cell constructs.

- 15 MOMP T-cell constructs MOMP_P3_ssHA1 (“P3”) and MOMP_P5_ssHA1 (“P5”) were designed using a structure-based strategy in order to replace VD1-4 regions (which contain B-cell epitopes).

As a basis for the structure guided design, Alphafold2 (Deepmind) was used to create a 3D model of the MOMP protein. The resulting MOMP model structure can be described as a beta-barrel made up of 10 antiparallel beta strands. The VD1-4 regions are situated in loops linking individual beta-strands, all located
20 on the same side of the barrel structure.

In order to design replacement sequences for the VD1-4 loops, each VD sequence was mapped onto the MOMP model structure. The distance between the start and end of each VD region was measured, and the approximate peptide sequence length needed to cover this distance between the start and end points of each VD was estimated.

- 25 The “Crosslink Proteins” (CS) protocol available through Maestro (v12.8, Schrodinger) was used to build peptide sequences of diverse lengths and amino-acid composition on the MOMP model structure, in order to replace the VD regions. After energy minimization and visual inspection of resulting MOMP structures, a shortlist of 6 MOMP VD-replaced sequences were selected for further analysis.

30 In order to avoid issues related to human cross reactivity for the newly designed VD-replaced MOMP sequences, sequence motifs of 8 or more amino acids in the VD-replaced MOMP sequences, which are also present in the human proteome, were excluded from potential designs.

Finally, a VD1-4-replaced MOMP sequence was selected in which the VD replacement peptides/loops are predicted to have minimal impact on the overall MOMP beta-barrel structure, and which has no sequence motif (8 or more amino acids) in common with sequences present in the human proteome.

The resulting T-cell constructs MOMP_P3_ssHA1 ("P3") and MOMP_P5_ssHA1 ("P5") are given by SEQ ID NOs: 53 and 57, respectively.

Design of B cell constructs (chimeric MOMP VD polypeptide constructs)

Further mRNA constructs encoding chimeric MOMP VD polypeptide antigens were prepared with the aim of eliciting MOMP-specific antibody (B cell) responses – i.e. MOMP B cell constructs.

The exemplary B cell constructs are chimeras which were designed by combining variable domains (VD) from 4 serovars of *C. trachomatis* (SerD, SerE, SerF & SerG). A chimeric antigen was obtained which combines copies of each of the 4 variable domains (VD1 to VD4) from different serovars, designed in this way in order to be able to induce a cross-serovar immune (antibody) response. A protein sequence comparison of the VD loops across serovars (SEQ ID NOs: 5 to 20) indicated that:

- VD1 loops of SerD and SerE are highly similar, and VD1 loops of SerF and SerG are identical
- VD2 loops of SerD and SerE are highly similar, and VD2 loops of SerF and SerG are highly similar
- VD3 loops of SerD and SerF are identical, and VD3 loops of SerF and SerG are highly similar
- VD4 loops of SerD and SerE are highly similar, and VD4 loops of SerF and SerG are highly similar

It was hypothesized that highly similar or identical sequences of VD loops between two serovars might be able to contribute to a cross-serovar response at least between those two serovars. Therefore, the designed chimeras contained two of each of VD1, VD2 and VD4 (i.e., 1 VD from D or E + 1 VD for F or G, for each of the VD loops). Due to a putative homology with a human protein the VD3 from SerE was excluded from the designs and only one VD3 (from SerG or SerD/F*) was included.

To maintain the VD loops in a conformation which is structurally similar to that of the VD loops within native MOMP, some of the flanking residues either side of the VD loops were included in the designed constructs. Two construct groups with different lengths of flanking sequences either side of the VD loops were designed (extP and extS).

Two construct groups with different combinations of VD loops were also designed (VDcomb1 and VDcomb2).

- VDcomb1 contains the following VD loops:
- VD1 (serD) – VD1 (serF/G*) – VD2 (serE) – VD2 (serF) – VD3 (serG) – VD4 (serE) – VD4 (serG)
- VDcomb2 contains the following VD loops:

VD1 (serE) – VD1 (serF/G*) – VD2 (serD) – VD2 (serG) – VD3 (serD/F*) – VD4 (serD) – VD4 (serF)

* the sequences for this particular VD from these serovars are identical

The resulting B cell constructs are given by protein sequences according to SEQ ID NO: 61-92.

EXAMPLE 2 – Expression of mRNA constructs

- 5 mRNA constructs encoding a selection of *C. trachomatis* (*Ct*) antigens were provided and expressed in HeLa cells as described in the Methods section.

Results

The following constructs were expressed and detected in lysate and/or supernatant, as indicated in Table 4 below. Expression in the supernatant indicated that the protein was secreted.

- 10 ssHA1 = signal peptide sequence of SEQ ID NO:187; ssHA2 = signal peptide sequence of SEQ ID NO:188; TMB1= transmembrane domain sequence of SEQ ID NO: 189; TMB2= transmembrane domain sequence of SEQ ID NO: 190; FL = full-length protein; C2S = variant containing cysteine to serine mutations which are designed to remove any disulphide bridges; GlycNeg = variant containing point mutations designed to prevent glycosylation; P3 and P5 = MOMP variants (T cell constructs) in which the variable loops (VDs)
- 15 have been replaced using a structure-based strategy according to the design strategy described in Example 1; VDcomb = chimeric MOMP variants (B cell constructs) in which variable loops (VDs) from different serovars are combined according to the design strategy described in Example 1; extP and extS= VDcomb construct which have different lengths of flanking sequences either side of the VD included (see Example 1 for design strategy); trunc = a truncated version of the relevant protein; CT812_pass-domain = CT812
- 20 fragment corresponding to amino acid residues 52-1003 of CT812 (SEQ ID NO: 515); CT812_ext_ pass-domain = CT812 fragment corresponding to amino acid residues 52-1179 of CT812 (SEQ ID NO: 515); HPXn-n_HPXn-n = constructs in which sequence motifs of 8 or more amino-acids, which are also present in the human proteome, were mutated in order to avoid human cross reactivity; N.D. = not determined.

Table 4 – mRNA constructs expressed in HeLa cells

SEQ ID NO	Construct Name	Lysate Expression	Supernatant Expression
Full Length MOMP			
879	MOMP_serD_FL_ssHA1	+++	+
294	MOMP_serD_FL_ssHA1	+++	+
297	MOMP_serD_FL_ssHA1_Glycneg	+++	+
298	MOMP_serD_FL_ssHA1_Glycneg	+++	+
873	MOMP_serD_FL_ssHA1_C2S_Glycneg	+++	+++
875	MOMP_serE_FL_ssHA1	+++	++

876	MOMP_serE_ssHA1_C2S_Glycneg	+++	+
880	MOMP_serF_FL_ssHA1	+++	+
326	MOMP_serF_FL_ssHA1	+++ (brighter)	+
338	MOMP_serF_FL_ssHA2_Glycneg	+++	+++
881	MOMP_serG_FL_ssHA1	+++	+
342	MOMP_serG_FL_ssHA1	+++ (brighter)	+
345	MOMP_serG_FL_ssHA1_Glycneg	+	+++
T Cell Constructs			
213	MOMP_P3_ssHA1	+ (Brighter)	not detected
218	MOMP_P3_ssHA1_C2S	+ (Brighter)	+
219	MOMP_P3_ssHA2_C2S	++	++
222	MOMP_P5_ssHA1	+++	+
225	MOMP_P5_ssHA1_C2S	+	+
B Cell Constructs			
229	MOMP_VDcomb1-extP_ssHA1	+++	+
863	MOMP_VDcomb1-extP_ssHA1	+++	+
877	MOMP_VDcomb1-extP_ssHA1_Glycneg	+++	++
232	MOMP_VDcomb1-extP_ssHA1_Glycneg	+++	+++
233	MOMP_VDcomb1-extP_ssHA1_TMB1	+	N.D.
234	MOMP_VDcomb1-extP_ssHA1_TMB1	+	N.D.
235	MOMP_VDcomb1-extP_ssHA1_TMB1_Glycneg	++	N.D.
865	MOMP_VDcomb1-extP_ssHA1_TMB1_Glycneg	++	N.D.
237	MOMP_VDcomb1-extP_ssHA2	+++	+
238	MOMP_VDcomb1-extP_ssHA2	+++	+
239	MOMP_VDcomb1-extP_ssHA2_Glycneg	+++	++
242	MOMP_VDcomb1-extP_ssHA2_TMB2	+	N.D.
243	MOMP_VDcomb1-extP_ssHA2_TMB2_Glycneg	++	N.D.
244	MOMP_VDcomb1-extP_ssHA2_TMB2_Glycneg	++	N.D.
245	MOMP_VD-comb1-extS_ssHA1	+++	not detected
864	MOMP_VD-comb1-extS_ssHA1	+++	+++
878	MOMP_VD-comb1-extS_ssHA1_Glycneg	+++	+++
248	MOMP_VD-comb1-extS_ssHA1_Glycneg	+++	+++
249	MOMP_VD-comb1-extS_ssHA1_TMB1	++	N.D.
251	MOMP_VD-comb1-extS_ssHA1_TMB1_Glycneg	++	N.D.
866	MOMP_VD-comb1-extS_ssHA1_TMB1_Glycneg	++	N.D.
253	MOMP_VD-comb1-extS_ssHA2	+++	+++
254	MOMP_VD-comb1-extS_ssHA2	+++	+++
255	MOMP_VD-comb1-extS_ssHA2_Glycneg	+++	+++
256	MOMP_VD-comb1-extS_ssHA2_Glycneg	+++	+++
257	MOMP_VD-comb1-extS_ssHA2_TMB2	++	N.D.
258	MOMP_VD-comb1-extS_ssHA2_TMB2	++	N.D.
259	MOMP_VD-comb1-extS_ssHA2_TMB2_Glycneg	++	N.D.
260	MOMP_VD-comb1-extS_ssHA2_TMB2_Glycneg	++	N.D.

261	MOMP_VDcomb2-extP_ssHA1	++	+
262	MOMP_VDcomb2-extP_ssHA1	++	+
868	MOMP_VDcomb2-extP_ssHA1_Glycneg	+++	++
264	MOMP_VDcomb2-extP_ssHA1_Glycneg	+++	+
265	MOMP_VDcomb2-extP_ssHA1_TMB1	+	N.D.
266	MOMP_VDcomb2-extP_ssHA1_TMB1	+	N.D.
871	MOMP_VDcomb2-extP_ssHA1_TMB1_Glycneg	++	N.D.
268	MOMP_VDcomb2-extP_ssHA1_TMB1_Glycneg	+	N.D.
269	MOMP_VDcomb2-extP_ssHA2	++	+
270	MOMP_VDcomb2-extP_ssHA2	++	+
869	MOMP_VDcomb2-extP_ssHA2_Glycneg	+++	++
272	MOMP_VDcomb2-extP_ssHA2_Glycneg	+++	++
273	MOMP_VDcomb2-extP_ssHA2_TMB2	+	N.D.
274	MOMP_VDcomb2-extP_ssHA2_TMB2	+	N.D.
867	MOMP_VDcomb2-extP_ssHA2_TMB2_Glycneg	+	N.D.
276	MOMP_VDcomb2-extP_ssHA2_TMB2_Glycneg	+	N.D.
277	MOMP_VDcomb2-extS_ssHA1	+++	+++
278	MOMP_VDcomb2-extS_ssHA1	+++	+++
870	MOMP_VDcomb2-extS_ssHA1_Glycneg	+++	+++
280	MOMP_VDcomb2-extS_ssHA1_Glycneg	+++	+++
872	MOMP_VDcomb2-extS_ssHA1_TMB1	+	N.D.
282	MOMP_VDcomb2-extS_ssHA1_TMB1	+	N.D.
283	MOMP_VDcomb2-extS_ssHA1_TMB1_Glycneg	++	N.D.
285	MOMP_VDcomb2-extS_ssHA2	+++	+++
287	MOMP_VDcomb2-extS_ssHA2_Glycneg	+++	+++
290	MOMP_VDcomb2-extS_ssHA2_TMB2	++	N.D.
291	MOMP_VDcomb2-extS_ssHA2_TMB2_Glycneg	++	N.D.
292	MOMP_VDcomb2-extS_ssHA2_TMB2_Glycneg	++	N.D.
Non-MOMP Constructs			
369	CT443_ssHA1 PolyA	+	not detected (band size is at albumin band)
370	CT443_ssHA1 PolyA	+	not detected (band size is at albumin band)
371	CT443_ssHA1_Glycneg PolyA	+	not detected (band size is at albumin band)
372	CT443_ssHA1_Glycneg PolyA	+	not detected (band size is at albumin band)
373	CT584_ssHA1 PolyA	+	+
374	CT584_ssHA1 PolyA	+++	+++
375	CT584_ssHA1_C2S PolyA	++	++
376	CT584_ssHA1_C2S PolyA	++	++
377	CT584_ssHA1_Glycneg PolyA	+++	+++

378	CT584_ssHA1_Glycneg PolyA	+++	+++
379	CT584_ssHA1_Glycneg_C2S PolyA	++	++
380	CT584_ssHA1_Glycneg_C2S PolyA	++	++
381	CT600_trunc_ssHA1	+++	+++
382	CT600_trunc_ssHA1	+++	++
383	CT600_trunc_ssHA1_Glycneg	+++	+++
384	CT600_trunc_ssHA1_Glycneg	+++	+++
385	CT812_pass-domain_ssHA1	++ (brighter)	not detected
386	CT812_pass-domain_ssHA1	++	not detected
387	CT812_pass-domain_ssHA1_C2S	++	not detected
388	CT812_pass-domain_ssHA1_C2S	++	not detected
389	CT812_ext-pass-domain_ssHA1	+	not detected
390	CT812_ext-pass-domain_ssHA1	+	not detected
391	CT812_ext-pass-domain_ssHA1_C2S PolyA	+	not detected
392	CT812_ext-pass-domain_ssHA1_C2S PolyA	+	not detected
393	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1 CO1	+++	+
394	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1 CO2	+++	+/-
395	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1_C2S CO1	+++	+/-
396	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1_C2S CO2	+++	+/-
397	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3_C2S CO1	+++	+
398	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3_C2S CO2	+++	+
399	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1 CO1	+++	+
400	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1 CO2	+++	+/-
401	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3 CO1	+++	+
402	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3 CO2	+++	+
403	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1 CO1	+++	+
404	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1 CO2	+++	+
405	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3 CO1	+++	+
406	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3 CO2	+++	+
407	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3_C2S CO1	+++	+/-
408	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3_C2S CO2	+++	+/-
409	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1_C2S CO1	+++	+
410	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1_C2S CO2	+++	+
411	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3 CO1	+++	+
412	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3 CO2	+++	+/-

413	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1_C2S CO1	+++	+/-
414	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1_C2S CO2	+++	+/-
415	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3_C2S CO1	+++	+
416	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3_C2S CO2	+++	+
417	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3_C2S CO1	+++	+
418	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3_C2S CO2	+++	+/-
419	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3 CO1	+++	+
420	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3 CO2	+++	+
421	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1 CO1	+++	+
422	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1 CO2	+++	+
423	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1_C2S CO1	+++	+
424	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1_C2S CO2	+++	+
Control Constructs			
862	Hirep2_ssHA1	+++	++
202	Hirep2_ssHA1	+++	+
365	CTH522_patent_ssHA1 [CO1] PolyA	+++	+
366	CTH522_patent_ssHA1 [CO2] PolyA	+++	+
367	CTH522_patent_ssHA1_Glycneg [CO1] PolyA	+++	+

Methods

HeLa cells were plated in 12-well plates at 0.15 million cells/well in 1 mL EMEM + 10% FBS. Cells were transfected the next day with 1 µg/well *Chlamydia* mRNA constructs with lipofectamine 2000.

5 Preparation of lysates

Cells were harvested the 22-24 hours later and lysed in 250µL per well of CellLytic M +1x HALT. Lysates were incubated on ice for 10 minutes then cleared in a microcentrifuge at max speed for 10min at 4°C. 15µL Lysate was combined with 5µL NuPAGE LDS Sample buffer with reducing agent. Reduced samples were incubated at 85°C for 5 minutes.

10 Preparation of supernatant samples

Supernatants were harvested the 22-24 hours after transfection. 15µL supernatant was combined with 5µL NuPAGE LDS Sample buffer with reducing agent. Reduced samples were incubated at 85°C for 5 minutes.

Analysis

The resulting lysate or supernatant samples were run on 8-16% Gradient SDS-PAGE at 185V for 60 minutes. Protein was transferred to nitrocellulose membrane. Blots were blocked with Intercept blocking buffer overnight at 4°C. Blots were stained with anti-*Chlamydia trachomatis* MOMP polyclonal antibody (for full-length MOMP constructs, T cell constructs, B cell constructs and control constructs), anti-CT443 polyclonal sera (for CT443 constructs), anti-CT584 polyclonal sera (for CT584 constructs), anti-CT600 polyclonal sera (for CT600 constructs) or anti-CT812 polyclonal sera (for CT812 constructs). Antibody staining was done in Intercept Blocking buffer + 0.2% Tween 20 for 2 hrs at room temperature (RT). Blots were washed with TBST 3x5minutes. Blots stained with anti-*Chlamydia trachomatis* MOMP polyclonal antibody were then stained with donkey anti-goat 800 secondary antibody in Intercept Blocking buffer + 0.2% Tween 20 for 1hr at RT. Blots stained with anti-CT443, anti-CT584, anti-CT600 or anti-CT812 polyclonal sera were then stained with donkey anti-rabbit 800 secondary antibody in Intercept Blocking buffer + 0.2% Tween 20 for 1hr at room temperature. Blots were washed with TBST 4x5minutes. Blots were scanned on Licor odyssey.

EXAMPLE 3 – Recombinant protein expression in *E. coli*

DNA constructs encoding *Ct* antigens were cloned into a T7 promoter-based expression vector for expression in *E. coli* using standard methods. *E. coli* (BL21 DE3) were transformed with the vector. Protein expression was induced with IPTG. The constructs were purified from inclusion bodies using nickel chelation chromatography, followed by protein refolding using the buffers outlined in Table 5.

Table 5: Constructs and composition of refolding buffers used for each construct

Construct	Refolding buffer
MOMP_P5_6his_coli (encoding protein of SEQ ID NO: 133)	50 mM Tris-HCl, 150 mM NaCl, 10% Glycerol, 0.2% SDS, pH 8.0
MOMP_serE_FL_Wt (encoding protein of SEQ ID NO: 134)	Refolding buffer containing 0.1% NLS detergent
MOMP_serE_FL_C2S_6his_coli (encoding protein of SEQ ID NO: 136)	20 mM Tris, 150 mM NaCl, 0.1% NLS
CT443_6his_coli (encoding protein of SEQ ID NO: 143)	PBS, 10% Glycerol, 500 mM NaCl, pH 7.4
6his_CT584_coli (encoding protein of SEQ ID NO: 145)	20 mM Tris, 150 mM NaCl, 0.1% NLS
6his_CT600_coli (encoding protein of SEQ ID NO: 146)	PBS, 0.2% NLS, pH7.4
CT812_ext-pass-domain_6his_coli (encoding protein of SEQ ID NO : 147)	PBS, 10% Glycerol, 0.2% Sodium Lauroyl Sarcosine, pH 7.4

The purified constructs were analysed using SDS-PAGE gel electrophoresis under reducing conditions. Western blotting using an anti-His tag antibody was performed for all the constructs except MOMP_serE_FL, and in each case detected the expressed product at the expected molecular weight on the

blot. SDS-PAGE under non-reducing conditions indicated that MOMP_serE_FL adopts a trimeric form. Construct stability and conformation were assessed by differential scanning calorimetry (DSC), and secondary structure was assessed using circular dichroism (CD).

EXAMPLE 4 – Immunisation with MOMP mRNA constructs elicits cross-serovar T cell responses in mice

Selected mRNA constructs encoding a native SS-MOMP delivering wild type (WT) full-length MOMP (“mRNA MNR MOMP E FL”; encoding protein of SEQ ID NO: 155) and MOMP without variable domains VD2 and VD4 (“mRNA UNR MOMP E DII:IV”; encoding protein of SEQ ID NO: 157) were tested for their ability to elicit T cell responses. SS-MOMP = native secretion signal sequence of *Ct* MOMP (SEQ ID NO: 192). As used herein, MNR = modified mRNA (in which all uridine nucleosides were replaced with 1-methyl pseudo uridine). UNR = unmodified mRNA (containing unmodified uridine nucleosides). As used herein in the examples, modified mRNAs (or MNRs) have the nucleic acid sequence as indicated by the SEQ ID NO wherein all uridine nucleosides have been replaced with 1-methylpseudouridine.

In the “mRNA UNR MOMP E DII:IV” construct, nucleic acid sequences encoding variable loops VD2 and VD4 were replaced with sequences encoding non-native loop motifs containing exclusively glycine residues, in order to remove any immunogenic epitopes contained within the loops.

Mice received two immunisations of the SS-MOMP constructs (2 µg dose), formulated with the LNP OF-02, given by IM route, at 0 and 3 weeks (W0 and W3). Two additional groups were injected with the “mRNA MNR MOMP E FL” construct according to the same methods but at 0.5µg and 0.125µg doses, respectively. 10 mice per group were immunised in each group. Studies included two negative controls: PBS (5 mice) or empty LNP OF-02 (10 mice). Splenocytes were collected fourteen days post-boost. The T specific cellular response was assessed by ELISPOT/FluoroSpot and by Intra cellular cell staining (ICCS).

LNP OF-02 (“Lipid A”) = cationic lipid OF-02 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%

Ability to elicit IFN γ -producing T cells (FluoroSpot)

First, ability to elicit IFN γ -producing T cells was tested using FluoroSpot. For the FluoroSpot assay, spleen cells (0.2×10^6 per well) were plated in 200 µl RPMI complete together with and without MOMP peptide pools from serovar D, E and F in presence of IL2 (Roche) in triplicate cultures each. The overlapping peptide pools used herein for restimulation consisted of 15-residue peptides covering the entire native protein sequence of interest with 11-residue overlaps between consecutive peptides. Subsequently, plates were incubated at 37°C, 5% CO₂ for 20 h. After washing with PBS, plates were incubated with detection Ab anti-IFN γ (mAb R4-6A2-BAM) for 2 h at RT. Image analysis of FluoroSpot assays was performed on Microvision Reader.

To assess the antigen specific response in an ELISPOT/FluoroSpot assay as described herein the number of cytokine producing spots are assessed after either incubation with a specific antigen (peptide, protein, whole bacteria) or no antigen. The average number of cytokine forming spots detected after an incubation with no antigen is considered the assay negative control and this typically very small number of spots is subtracted from the number of cytokine forming spots formed after the different antigen stimulations. This is how the ELISPOT/FluoroSpot data is normalized. Thus, in an ELISPOT/FluoroSpot assay as described herein cells are plated together with and without peptide pools, assessing both the response to antigen stimulation (with peptide pools) and no antigen stimulation (without peptide pools). The “without” peptide data are used to normalize the antigen specific data shown in the corresponding Figures.

As shown in Figure 1, both “mRNA MNR MOMP E FL” and “mRNA UNR MOMP E DII:IV” (2 µg) were able to induce a comparable level of cross-serovar-responsive IFN γ -producing T cells, suggesting that the T cell response is maintained despite removal of VD loops. Responses were dose-dependent, with little observable difference between 2 µg and 0.5 µg. A 0.125µg dose was therefore used in later studies comparing mRNA constructs.

Ability to elicit CD4+IFN γ + and CD8+IFN γ + T cell responses (ICCS)

The MOMP mRNA constructs were also tested for their ability to elicit CD4+IFN γ + T cells and CD8+IFN γ + T cells in mice that are reactive across various serovars.

For ICCS, isolated lymphocytes were activated with specific serovar E- and F MOMP peptide pools (as described above) at 1×10^6 cells/well in 96-well plates and cultured for 6 h in 5% CO $_2$ at 37°C with BFA in order to block cytokine secretion. Activated cells were harvested for surface and intracellular cytokine staining. Surface staining of the cells was performed by incubating with fluorescent conjugated monoclonal antibodies specific to CD3, CD4, and CD8 for 20 min on ice. After surface staining, the cells were washed two times, fixed and permeabilized for 20 min at 4°C using BD Cytofix/Cytoperm buffer (BD Biosciences) and washed twice with the BD Perm/Wash buffer. Intracellular cytokine staining was then performed by incubating with the fluorescent conjugate antibodies against murine IL-2, IL-5, IL-10, TNF- α , and IFN- γ on ice for 30 min. The cells were washed three times and analyzed with a FACS Fortessa flow cytometer (BD Biosciences).

As shown in Figure 2A, both “mRNA MNR MOMP E FL” and “mRNA UNR MOMP E DII:IV” (2 µg) were able to induce cross-serovar-responsive CD4+IFN γ + T cells. Responses for “mRNA MNR MOMP E FL” (tested at multiple doses) were dose-dependent.

As shown in Figure 2B, “mRNA MNR MOMP E FL” induced cross-serovar-responsive CD8+IFN γ + cells in a dose-dependent manner. “mRNA UNR MOMP E DII:IV” (2 µg dose) elicited minimal CD8+IFN γ + T cell responses.

Thus, removal of VD loops may elevate CD4+IFN γ + T cell responses.

EXAMPLE 5 – Immunisation with MOMP mRNA elicits an immune response

LNP-formulated constructs (all unmodified mRNAs) were used to immunise mice. 8 mice (Balb/c) were immunised in each study group. The following study groups were assessed (human leader = signal sequence of SEQ ID NO: 191):

- Group 1: Naïve – Negative control (LNP (MC3) buffer)
- Group 2: UV EB + CpG prime and live boost (First priming immunisation with *Ct* elementary bodies (EB) (10^7 inclusion-forming units (IFU) of UV-inactivated EB) + 20 μ g CpG adjuvant, and second boost immunisation with 10^7 IFU live *Ct* serovar E EB + 20 μ g CpG adjuvant)
- Group 3: rMOMP (recombinant serovar E MOMP protein) (SEQ ID NO: 134); 50 μ g dose + 20 μ g CpG adjuvant
- Group 4: MC3 Ser E MOMP (encoding protein of SEQ ID NO: 148); 5 μ g dose
- Group 5: MC3 Ser E MOMP (encoding protein of SEQ ID NO: 148); 0.5 μ g dose
- Group 6: MC3 Ser E MOMP with human leader (encoding protein of SEQ ID NO: 149); 5 μ g dose
- Group 7: MC3 Ser E MOMP with human leader (encoding protein of SEQ ID NO: 149); 0.5 μ g dose
- Group 8: OF-02 Ser E MOMP (encoding protein of SEQ ID NO: 151); 5 μ g dose
- Group 9: OF-02 Ser E MOMP (encoding protein of SEQ ID NO: 151); 0.5 μ g dose
- Group 10: OF-02 Ser E MOMP with human leader (encoding protein of SEQ ID NO: 152); 5 μ g dose
- Group 11: OF-02 Ser E MOMP with human leader (encoding protein of SEQ ID NO: 152); 0.5 μ g dose

Immunisations were given at days 0 and 28, and bleeds were performed at days 0, 14, 28 and 42. Spleens were collected at day 42.

Ability to elicit IgG that binds recombinant MOMP (ELISA)

Reciprocal dilution IgG titers were assessed in plate ELISA using recombinant serovar E MOMP. The formulation with OF-02 LNP led to higher titers at day 42 than the formulation with MC3 (Figure 3). Titers for OF-02-formulated constructs at 5 μ g were at levels comparable to those of the EB + CpG and rMOMP + CpG controls.

Ability to elicit IFN γ -producing T cells (ELISPOT)

T cell responses were assessed in splenic IFN γ ELISPOT, as shown in Figures 4A, B and C. Different recall antigens were used, and are indicated above the individual graphs: “UV EB”, which stands for UV inactivated EBs (Figure 4A), “rMOMP”, which is recombinant serovar E MOMP protein (Figure 4B), and “total peptide pool”, which stands for a pool of overlapping serovar E MOMP peptides (as described above) (Figure 4C).

These data indicate that immunisation with either OF-02 or MC3 formulated WT serovar E MOMP mRNA elicits MOMP specific IFN γ -producing T cells, and these T cells are elicited in response to any of the tested recall antigens.

EXAMPLE 6 – MOMP constructs lacking one or more VD loops induce a T cell response

Further mRNA (UNR) constructs delivering MOMP without variable domains were tested for their ability to elicit T cell responses.

The removal of the variable domains does not impair the ability to elicit an IFN γ MOMP T cell response

In this example, mice (female C57BL/6) were immunised with MOMP mRNA constructs (containing unmodified mRNA), or a recombinant MOMP control, to test the effects of the systematic removal of variable domain loops VD1, VD2, VD3, VD4 from MOMP serovar E, either alone or in combination. In the Δ VD constructs, nucleic acid sequences were modified so that variable loops were replaced with non-native loop motifs containing glycine residues, in order to remove any immunogenic epitopes contained within the loops. For example, Δ VD1 encodes a serovar E MOMP in which the VD1 loop is replaced by six Gly residues. All of the mRNA constructs also encoded the native signal sequence (of SEQ ID NO: 192), with exception of the Δ -signal-peptide mRNA construct.

Two studies as set out in the tables below (Table 6 and Table 7) were performed, and the data presented in Figures 5A and B, respectively show analysis of splenocytes from mice in these groups:

Table 6 – Study groups presented in Figure 5A

Group N=10	Antigen (mRNA)	Dose (μ g)	LNP or Adjuvant	Dosing schedule (days)	Bleeding schedule (days)
1	Wild type MOMP (encoding protein of SEQ ID NO: 154)	2	OF-02	0, 21	Pre-immunisation - 1 (Pre-), 21, 34
2	Δ VD4 (encoding protein of SEQ ID NO: 156)	2	OF-02	0, 21	Pre-, 21, 34
3	Δ VD2 Δ VD4 (encoding protein of SEQ ID NO: 157)	2	OF-02	0, 21	Pre-, 21, 34
4	Δ VD1 Δ VD2 Δ VD4 (encoding protein of SEQ ID NO: 158)	2	OF-02	0, 21	Pre-, 21, 34

5	ΔVD1234 (encoding protein of SEQ ID NO: 159)	2	OF-02	0, 21	Pre-, 21, 34
6	Δ-signal-peptide (encoding protein of SEQ ID NO: 160)	2	OF-02	0, 21	Pre-, 21, 34
Protein Controls					
7	rMOMP (SEQ ID NO: 134)	5	AF03	0, 21	Pre-, 21, 34

Table 7 – Study groups presented in Figure 5B

Group N=5	Antigen (mRNA)	Dose (ug)	LNP or Adjuvant	Dosing schedule (days)	Bleeding schedule (days)
1	ΔVD1 (encoding protein of SEQ ID NO: 161)	2	OF-02	0, 21	Pre-, 21, 35
2	ΔVD2 (encoding protein of SEQ ID NO: 162)	2	OF-02	0, 21	Pre-, 21, 35
3	ΔVD3 encoding protein of (SEQ ID NO: 163)	2	OF-02	0, 21	Pre-, 21, 35
4	ΔVD2 ΔVD3 (encoding protein of SEQ ID NO: 164)	2	OF-02	0, 21	Pre-, 21, 35
5	ΔVD1 ΔVD2 ΔVD3 (encoding protein of SEQ ID NO: 165)	2	OF-02	0, 21	Pre-, 21, 35
6	ΔVD2 ΔVD3 ΔVD4 (encoding protein of SEQ ID NO: 166)	2	OF-02	0, 21	Pre-, 21, 35
7	ΔVD1 ΔVD3 ΔVD4 (encoding protein of SEQ ID NO: 167)	2	OF-02	0, 21	Pre-, 21, 35

The ELISPOT assay was used to evaluate the T cell response of the mice after intramuscular (IM) injection, by measuring the level of IFN γ production by the T cells collected from the spleens. Spleens from individual mice were collected on the day of the final bleed (34/35) from the mice and T cell responses in splenocytes were assessed by ELISPOT, with pools of overlapping peptides of serovar E MOMP (as described above) used as recall antigen to stimulate the T cell response.

Figure 5A and B show the level of IFN γ production by the T cells following immunisation with mRNA constructs and stimulation with recall antigen. The data indicate that removal of the signal sequence eliminates the T cell response.

Immunisation with mRNA encoding MOMP serovar E with one or more of the variable domain loops VD1, VD2, VD3 and VD4 removed, and various combinations thereof, gives rise to a similar, if not enhanced, T cell response to that of FL MOMP serovar E. Therefore, removal of the variable domains does not impair ability to elicit an IFN γ MOMP T cell response.

MOMP without variable loops VD2 and VD4 is able to elicit an immune response

Unmodified (UNR) mRNA encoding MOMP without DII (VD2) and DIV (VD4) domain (DIIDIV), “mRNA UNR MOMP E DII:IV” (encoding protein of SEQ ID NO: 157), was compared to recombinant

MOMP protein (SEQ ID NO: 134) for its ability to induce a T cell response. In the "mRNA UNR MOMP E DII:IV" construct, variable loops VD2 and VD4 were replaced with non-native loop motifs containing glycine residues, in order to remove any immunogenic epitopes contained within the loops.

5 Mice (N=15 in each group) received two immunisations of unmodified (UNR) MOMP deleted of DII and DIV domain (DIIDIV) mRNA native SS-constructs (native sequence signal) ("mRNA UNR MOMP E DII:IV") and recombinant MOMP protein (serE) at 2 and 5 µg dose respectively, formulated with the LNP OF-02 or with a liposome-based adjuvant containing TLR4 agonist (SPA14, as described in WO2022090359) respectively, given by IM route, at 0 and 3 weeks (W0 and W3). A control group (N=15) was injected with PBS. Studies included a negative PBS control. Splenocytes were collected seven, eleven-
10 and fourteen-days post-boost. T specific cellular response was assessed by ELISPOT/FluoroSpot and by ICCS.

Ability to elicit IFN γ -producing T cells (FluoroSpot)

First, ability to elicit IFN γ -producing T cells was tested using FluoroSpot. For the FluoroSpot assay, spleen cells (0.2, 0.5 and 1 x 10⁶ per well) were plated in 200 µl RPMI complete together with and without MOMP
15 peptide pools (as described above) from serovar E in presence of IL2 (Roche) in triplicate cultures each. Subsequently plates were incubated at 37°C, 5% CO₂ for 20 h. After washing with PBS, plates were incubated with detection Ab anti-IFN γ (mAb R4-6A2-BAM) for 2h at RT. Image analysis of FluoroSpot assays was performed on Microvision Reader.

As shown in Figure 6A, MOMP DIIDIV UNR mRNA (i.e. "mRNA UNR MOMP E DII:IV") was able to
20 induce a comparable level of IFN γ -producing T cells relative to recombinant MOMP protein as formulated with adjuvant. High levels of IFN γ -producing T cells were observed between these antigens at days 7, 11 and 14. Therefore, removal of the variable domains VD2 and VD4 does not impair ability to elicit an IFN γ MOMP T cell response relative to rMOMP formulated with SPA14 adjuvant.

Ability to elicit CD4+IFN γ + and CD8+IFN γ + cell responses (ICCS)

25 The "mRNA UNR MOMP E DII:IV" construct was also compared to recombinant MOMP protein for its ability to elicit CD4+IFN γ + cells and CD8+IFN γ +cells, using ICCS.

For ICCS, isolated lymphocytes from the D7, D11 and D14 splenocyte samples were activated with specific serovar E-MOMP peptide pool (as described above) at 1 x 10⁶ cells/well in 96-well plates and cultured for 6 h in 5% CO₂ at 37°C with BFA in order to block cytokine secretion. Activated cells were harvested for
30 surface and intracellular cytokine staining. Surface staining of the cells was performed by incubating with fluorescent conjugated monoclonal antibodies specific to CD3, CD4, and CD8 for 20 min on ice. After surface staining, the cells were washed two times, fixed and permeabilized for 20 min at +4°C using BD Cytofix/Cytoperm buffer (BD Biosciences) and washed twice with the BD Perm/Wash buffer. Intracellular

cytokine staining was then performed by incubating with the fluorescent conjugate antibodies against murine IL-2, IL-5, IL-10, TNF- α , and IFN γ on ice for 30 min. The cells were washed three times and analyzed with a FACS Fortessa flow cytometer (BD Biosciences).

As shown in Figure 6B, across each of the splenocyte samples (collected 7, 11 and 14 days post-boost, respectively), both MOMP DIIDIV UNR mRNA (i.e. “mRNA UNR MOMP E DII:IV”) and recombinant MOMP protein were able to elicit the production of CD4+ IFN γ cells. As shown in Figure 6C, MOMP DIIDIV UNR mRNA also elicited a CD8+ IFN γ cell response, whereas recombinant MOMP protein did not.

In conclusion, the MOMP DIIDIV UNR mRNA, like rMOMP, elicits a significant CD4+ IFN γ cell response. Additionally, it shows a CD8+ IFN γ cell response which is not present for rMOMP.

EXAMPLE 7 – T cell constructs elicit a strong T cell response

T cell constructs are able to elicit a T cell response

7.1 The following mRNA constructs of MOMP variants (including those designed in Example 1; all modified) were formulated in LNP OF-02 and tested for their ability to produce a T cell response:

- MOMP_P3_ssHA1 (“P3”) (encoding protein of SEQ ID NO: 53)
- MOMP_P5_ssHA1_C2S (“P5 C2S”) (encoding protein of SEQ ID NO: 59)
- MOMP_serE_FL_ssHA1 (“FL”) (encoding protein of SEQ ID NO: 29)
- MOMP_serE_FL_ssHA1_C2S_Glycneg (“FL C2S Glycneg”) (encoding protein of SEQ ID NO: 35)
- Empty LNP OF-02

Mice received two immunisations of mRNA ssHA1-constructs (comprising sequence signal from hemagglutinin H1N1 A/Caledonia/20/1999; ssHA1 is given by SEQ ID NO: 187) at 0.125 μ g dose, formulated with the LNP OF-02, given by IM route, at 0 and 3 weeks (W0 and W3). Studies included a negative empty-LNP control and a positive full-length-MOMP-Serovar E control. Splenocytes were collected two weeks post-boost. A T specific cellular response was assessed by ELISPOT/ FluoroSpot and by ICCS.

7.2 In a second study, the following mRNA constructs of MOMP variants (including those designed in Example 1; all modified) were formulated in LNP OF-02 and tested for their ability to produce a T cell response:

- MOMP_P3_ssHA1 (“P3”) (encoding protein of SEQ ID NO: 53)
- MOMP_P3_ssHA1_C2S (“P3 C2S”) (encoding protein of SEQ ID NO: 55)
- MOMP_P5_ssHA1 (“P5”) (encoding protein of SEQ ID NO: 57)
- MOMP_P5_ssHA1_C2S (“P5 C2S”) (encoding protein of SEQ ID NO: 59)

- MOMP_serE_FL_ssHA1 (“Ser E FL”) (encoding protein of SEQ ID NO: 29)
- MOMP_serD_FL_ssHA1 (“Ser D FL”) (encoding protein of SEQ ID NO: 21)
- Empty LNP OF-02

Mice received two immunisations of mRNA ssHA1-constructs (comprising sequence signal from hemagglutinin H1N1 A/Caledonia/20/1999; ssHA1 is given by SEQ ID NO: 187) at 0.125 µg or 2 µg dose, formulated with the LNP OF-02, given by IM route, at 0 and 3 weeks (W0 and W3). Studies included a negative empty-LNP control and positive full-length-MOMP-Serovar E and full-length-MOMP-Serovar D controls. Splenocytes were collected one week post-boost. A T specific cellular response was assessed by ELISPOT/ FluoroSpot (0.125 µg dose) and by ICCS (2 µg dose). Results provided in this Example were obtained from the study described in point 7.1, unless indicated otherwise.

Ability to elicit IFN γ -producing T cells and IL-5 response (FluoroSpot)

First, ability to elicit IFN γ -producing and IL-5 producing T cells was tested using FluoroSpot. For the FluoroSpot assay, spleen cells (2×10^5 per well) were plated in 200 µl RPMI complete together with and without MOMP peptide pools (as described above) from serovar D, E, F, G and J (left to right / light grey to dark grey, respectively in Figures 7-8) in the presence of IL2 (Roche) and in triplicate cultures. Subsequently, plates were incubated at 37°C, 5% CO₂ for 20 h. After washing with PBS, plates were incubated with detection Ab anti-IFN γ (mAb R4-6A2-BAM), or detection Ab anti-IL-5 (mAb TRFK4, biotin) for 2 h at RT. Image analysis of FluoroSpot assays was performed on Microvision Reader.

As shown in Figure 7, MOMP_P3_ssHA1 (“P3”) and MOMP_P5_ssHA1_C2S (“P5 C2S”), like MOMP_serE_FL_ssHA1 (“FL”), gave rise to high numbers of cross-serovar-responsive IFN γ -producing T cells. In contrast, MOMP_serE_FL_ssHA1_C2S_Glycneg (“FL C2S Glycneg”) did not elicit a level of cross-serovar-responsive IFN γ -producing T cell response above that of empty LNPs.

As shown in Figure 8, all of the immunisation samples (MOMP_P3_ssHA1 (“P3”), MOMP_P5_ssHA1_C2S (“P5 C2S”), MOMP_serE_FL_ssHA1 (“FL”), MOMP_serE_FL_ssHA1_C2S_Glycneg (“FL C2S Glycneg”) and empty LNP) induced low levels of cross-serovar-responsive IL-5-producing cells, indicating a minimal Th2 response.

mRNA constructs evaluated in the study detailed in section 7.2 were also tested for their ability to elicit IFN γ -producing T cells using the FluoroSpot assay described above with plating of 1×10^5 spleen cells per well.

As shown in Figure 9, all mRNA constructs tested in the study detailed in section 7.2 gave rise to high numbers of cross-serovar-responsive IFN γ -producing T cells.

Ability to elicit CD4+IFN γ +, CD8+IFN γ +, and polyfunctional cell responses (ICCS)

The MOMP mRNA constructs were also tested for their ability to elicit CD4+IFN γ + cells, CD8+IFN γ + cells and polyfunctional cells in mice.

For ICCS, isolated lymphocytes were activated with specific serovar E-MOMP peptide pool (as described above) at 1×10^6 cells/well in 96-well plates and cultured for 6 h in 5% CO₂ at 37 °C with BFA in order to block cytokine secretion. Activated cells were harvested for surface and intracellular cytokine staining. Surface staining of the cells was performed by incubating with fluorescent conjugated monoclonal antibodies specific to CD3, CD4, and CD8 for 20 min on ice. After surface staining, the cells were washed two times, fixed and permeabilized for 20 min at +4°C using BD Cytofix/Cytoperm buffer (BD Biosciences) and washed twice with the BD Perm/Wash buffer. Intracellular cytokine staining was then performed by incubating with the fluorescent conjugate antibodies against murine IL-2, IL-5 or IL-17, IL-10, TNF- α , and IFN γ on ice for 30 min. The cells were washed three times and analyzed with a FACS Fortessa flow cytometer (BD Biosciences).

As shown in Figure 10A, MOMP_P3_ssHA1 (“P3”) and MOMP_P5_ssHA1_C2S (“P5 C2S”), like MOMP_serE_FL_ssHA1 (“FL”), gave rise to significant CD4+ IFN γ T cell responses. In contrast, MOMP_serE_FL_ssHA1_C2S_Glycneg (“FL C2S Glycneg”) did not give rise to a CD4+ IFN γ T cell response that was distinguishable from the empty LNP control.

As shown in Figure 10B, CD8+ IFN γ T cell responses were absent or much lower for the tested constructs.

The MOMP mRNA constructs were also assessed for their ability to induce polyfunctional cells. Figure 12 shows, for each of the mRNA constructs, the percentage of MOMP-specific CD4+ T cells that were elicited and indicates the relative percentages of CD4+ T cells producing each of IFN γ (“IFN γ ”), IL2, and/or TNF α (singularly and in the various combinations thereof). For each of MOMP_P3_ssHA1 (“P3”), MOMP_P5_ssHA1_C2S (“P5 C2S”) and MOMP_serE_FL_ssHA1 (“FL”), the CD4+IFN γ +IL2+TNF α + cell population formed the majority, relative to the other cell populations. Little to no IL5 or IL10 was observed for any of the tested constructs (data not shown).

The MOMP mRNA constructs evaluated in the study described in section 7.2, were similarly tested for their ability to elicit MOMP-specific CD4+IFN γ + cells, CD8+IFN γ + T cells and polyfunctional cells in mice.

As shown in Figure 11A, all MOMP mRNA constructs gave rise to a significant CD4+ IFN γ T cell response that was distinguishable from the empty LNP control. The CD4+ IFN γ T cell response was slightly higher for MOMP_P3_ssHA1 (“P3”), MOMP_P5_ssHA1 (“P5”), MOMP_P3_ssHA1_C2S (“P3 C2S”), and MOMP_P5_ssHA1_C2S (“P5 C2S”) as compared to the full length MOMP mRNA constructs.

As shown in Figure 11B, CD8+ IFN γ T cell responses were absent or much lower for the tested constructs, with the exception of the MOMP_P5_ssHA1_C2S (“P5 C2S”) and MOMP_serD_FL_ssHA1 (“SerD FL”) constructs.

5 The MOMP mRNA constructs evaluated in the study described in section 7.2, were also assessed for their ability to induce polyfunctional cells. Figure 13 shows, for each of the MOMP mRNA constructs, the percentage of MOMP-specific CD4+ T cells that were elicited and indicates the relative percentages of CD4+ T cells producing each of IFN γ (“IFN γ ”), IL2, and/or TNF α (singularly and in the various combinations thereof). For all of the MOMP mRNA constructs tested, the CD4+IFN γ +IL2+TNF α + cell population formed the majority, relative to the other cell populations. Little to no IL-17 or IL-10 was
10 observed for any of the tested constructs (data not shown).

T cell constructs elicit low levels of rMOMP binding antibodies

The MOMP mRNA constructs were also evaluated to determine if they elicited recombinant MOMP (rMOMP) binding antibodies. Blood samples were collected at 0 weeks (W0) and one week after the last vaccine administration, according to the protocol described in section 7.2 above, in order to measure total
15 IgG by ELISA. The specific IgG were measured from individual sera using automated 384 ELISA.

Briefly, 384-well micro-plates were coated in carbonate buffer with 20 μ L per well recombinant MOMP protein at 2 μ g/mL and kept overnight at +4°C. Coating solution was removed and washed with buffer 1 (PBS/Tween 0.05%). Free sites were blocked with 75 μ L of buffer 2 (PBS / Tween 20 at 0.05% / milk 1%) and incubated 90 min at room temperature (RT). Plates were emptied, then sera were serially diluted in
20 buffer 2 under a volume of 20 μ L (12 times) in the microplates. The plates were incubated for 90 min at RT and then washed with buffer 1. 20 μ L of a diluted anti-mouse total IgG peroxidase conjugate (Southern Biotech) was added in each well (1/2000). After 90 min incubation at RT, the plates were washed with buffer 1. The reaction was developed by adding 20 μ L of a tetramethylbenzidine substrate solution in each well. The reaction was chemically stopped after 30 min at RT with HCl (1N (normality)) and absorbance
25 was measured at 450-650 nm on a spectrophotometer (Synergy HTX, Biotek). The results were analysed in Softmax Pro software using a standard curve and expressed in arbitrary ELISA units by the reciprocal dilution corresponding to an OD of 1.

As shown in Figure 14, the MOMP mRNA constructs (“P3”, “P3 C2S”, “P5”, and “P5 C2S”) tested elicited low levels of recombinant MOMP binding antibodies. Levels were similar to those observed in pre-immune
30 sera or with empty LNPs, as expected given that these constructs lacked variable domain loops.

EXAMPLE 8 – Immunisation with non-MOMP antigens induces immune responses

Non-MOMP Ct protein constructs induce a T cell response

Mice (female C57BL/6) were immunised with various Ct non-MOMP recombinant proteins, alongside controls of recombinant MOMP protein or MOMP ΔVD1234 mRNA. The study groups are set out in Table 8 below:

Table 8 – Study groups

Group N=5	Antigen	Dose (ug)	Administration	Adjuvant	Dosing schedule (days)	Bleeding schedule (days)
1	rCT584; SEQ ID NO: 145	2	Subcutaneous (SC)	10 mg CpG/mouse/ injection +IFA	0, 21	Pre-, 21, 37
2	rCT812; SEQ ID NO: 197	2	SC	10 mg CpG/mouse/ injection +IFA	0, 21	Pre-, 21, 37
3	rCT600; SEQ ID NO: 146	2	SC	10 mg CpG/mouse/ injection +IFA	0, 21	Pre-, 21, 37
4	rCT443; SEQ ID NO: 198	2	SC	10 mg CpG/mouse/ injection +IFA	0, 21	Pre-, 21, 37
5	Viable EB (E/Bour ATCC VR-348B)	1e7*	SC	10 mg CpG/mouse/ injection +IFA	0, 21	Pre-, 21, 37
6	rMOMP; SEQ ID NO: 134	2	SC	10 mg CpG/mouse/ injection +IFA	0, 21	Pre-, 21, 37
7	MOMP ΔVD1234 (UNR mRNA) (encoding protein of SEQ ID NO: 159)	2	Intramuscular (IM)	OF-02	0, 21	Pre-, 21, 37

The ELISPOT assay was used to evaluate the T cell response of the mice after immunisation, by measuring the level of IFNγ production produced by the T cells collected from the spleens. Spleens from individual mice were collected from the mice and T cell responses in splenocytes were assessed by ELISPOT, with and without pools of overlapping peptides as described above of the relevant specified native MOMP or non-MOMP used as recall antigen to stimulate the T cell response.

As shown in Figure 15, recombinant (r) non-MOMP proteins “rCT584”, “rCT812”, “rCT600” and “rCT443” each induce IFNγ-producing T cells at much greater levels than EB. Responses elicited by recombinant MOMP protein (“rMOMP”; SEQ ID NO: 134) and mRNA encoding MOMP serovar E lacking VD loops 1-4 (“mRNA MOMP ΔVD1234”) are included for comparison.

mRNA constructs encoding non-MOMP Ct antigens induce a T cell response

Mice (female C57BL/6) were immunised (IM) with unmodified (UNR) mRNA encoding various *Ct* non-MOMP antigen fragments alongside the “mRNA MOMP ΔVD1234” construct, formulated in cKK-e10-based LNPs. LNP cKK-e10 (“Lipid B”) = cationic lipid cKK-e10 at a molar ratio of 40%; DMG-PEG2000 at a molar ratio of 1.5%; cholesterol at a molar ratio of 28.5%; and DOPE at a molar ratio of 30%.

The study groups are set out in Table 9 below:

Table 9 – Study groups

Group N=5	Antigen (all unmodified mRNA)	Dose (ug)	LNP	Dosing schedule	Bleeding schedule
1	CT812: 1-1530 (encoding protein of SEQ ID NO: 168)	2	cKK-e10	0, 21	Pre-, 21, 35
2	CT812: 1-761 (encoding protein of SEQ ID NO: 169)	2	cKK-e10	0, 21	Pre-, 21, 35
3	Nan96-SS:CT812: 32-761 (encoding protein of SEQ ID NO: 170)	2	cKK-e10	0, 21	Pre-, 21, 35
4	CT600: 1-188 (encoding protein of SEQ ID NO: 171)	2	cKK-e10	0, 21	Pre-, 21, 35
5	Nan96-SS:CT600: 2-188 (encoding protein of SEQ ID NO: 172)	2	cKK-e10	0, 21	Pre-, 21, 35
6	CT443: 1-576 (encoding protein of SEQ ID NO: 173)	2	cKK-e10	0, 21	Pre-, 21, 35
7	Nan96-SS:CT443: 32-576 (encoding protein of SEQ ID NO: 174)	2	cKK-e10	0, 21	Pre-, 21, 35
8	MOMP ΔVD1234 (encoding protein of SEQ ID NO: 159)	2	cKK-e10	0, 21	Pre-, 21, 35

Nan96-SS is the ssHA1 secretion signal given by SEQ ID NO:187. Numbering after the colons indicates the start and end residue (numbering relative to wild-type) of the relevant fragment of each of non-MOMP proteins CT443, CT600, and CT812. In the Nan96-SS constructs, the nucleic acid sequences encoding the ssHA1 secretion signal of SEQ ID NO: 187 and the non-MOMP protein fragment are linked by a nucleic acid sequence encoding the amino acids DTI.

The ELISPOT assay was used to evaluate the T cell response of the mice after immunisation, by measuring the level of IFNγ production produced by the T cells collected from the spleens. Spleens from individual mice were collected from the mice (day 35) and T cell responses in splenocytes were assessed by ELISPOT, with pools of overlapping peptides to the relevant specified MOMP or non-MOMP (as described above) used as recall antigen to stimulate the T cell response.

As shown in Figure 16, all of the non-MOMP fragment mRNA constructs (CT812: 1-1530, CT812: 1-761, Nan96-SS:CT812: 32-761, CT600: 1-188, CT443: 1-576, Nan96-SS:CT443: 32-576), gave rise to comparable levels of IFN γ -producing T cells to the mRNA encoding MOMP serovar E lacking VD loops 1-4 ("MOMP Δ VD1234"), with exception of Nan96-SS:CT600: 2-188.

- 5 Therefore, various mRNA constructs encoding non-MOMP *Ct* antigen fragments are also able to elicit a significant IFN γ -producing T cell response.

mRNA constructs encoding non-MOMP *Ct* antigens induce a T cell response

- mRNA encoding non-MOMP *Ct* antigens "CT443" (Nan96-SS:CT443: 32-576), "CT584" (Nan96HA-SS:CT584:2-183), "CT600" (Nan96HA-SS:CT600:2-188) and "CT812" (Nan96-SS:CT812:32-761) were
10 compared to recombinant MOMP protein for their ability to induce a T cell response.

- Mice received two immunisations of unmodified mRNA native signal sequence constructs at 2 μ g dose, formulated with the cKK-e10-based LNP, given by IM route, at 0 and 3 weeks (W0 and W3). Study included a positive full-length-MOMP-Serovar E recombinant protein control (SEQ ID NO: 134) (5 μ g) + SPA14. Splenocytes were collected at days eleven and fourteen post-boost. T specific cellular response was
15 assessed by ELISPOT/FluoroSpot and by ICCS.

Ability to elicit IFN γ -producing T cells (FluoroSpot)

- First, ability to elicit IFN γ -producing T cells was tested using FluoroSpot. For the FluoroSpot assay, spleen cells (2×10^5 per well) were plated in 200 μ l RPMI complete together with and without respective peptide pools (as described above) in presence of IL2 (Roche) at 0.5 to 2 μ g/mL in triplicate cultures each.
20 Subsequently plates were incubated at 37°C, 5% CO $_2$ for 20 h. After washing with PBS, plates were incubated with detection Ab anti-IFN γ (mAb R4-6A2-BAM) for 2 h at RT. Image analysis of FluoroSpot assays was performed on Microvision Reader.

- As shown in Figure 17, mRNA encoding non-MOMP *Ct* antigens CT443, CT584 and CT812 were, like recombinant MOMP serovar E protein, able to elicit high levels of IFN γ -producing T cells. Responses to
25 mRNA encoding non-MOMP *Ct* antigen CT600 were lower.

Therefore, various mRNA constructs encoding native non-MOMP *Ct* antigens (particularly CT443, CT584 and CT812) are able to elicit a strong IFN γ -producing T cell response.

Ability to elicit CD4+IFN γ + and CD8+IFN γ + cell responses (ICCS)

- The same constructs were also tested for their ability to elicit CD4+IFN γ + cells and CD8+IFN γ +cells in
30 mice.

For ICCS, isolated lymphocytes were activated with specific respective peptide pools (as described above) at 1×10^6 cells/well in 96-well plates and cultured for 6 h in 5% CO $_2$ at 37 °C with BFA in order to block

cytokine secretion. Activated cells were harvested for surface and intracellular cytokine staining. Surface staining of the cells was performed by incubating with fluorescent conjugated monoclonal antibodies specific to CD3, CD4, and CD8 for 20 min on ice. After surface staining, the cells were washed two times, fixed and permeabilized for 20 min at +4°C using BD Cytofix/Cytoperm buffer (BD Biosciences) and washed twice with the BD Perm/Wash buffer. Intracellular cytokine staining was then performed by incubating with the fluorescent conjugate antibodies against murine IL-2, IL-5, IL-10, TNF- α , and IFN γ on ice for 30 min. The cells were washed three times and analyzed with a FACS Fortessa flow cytometer (BD Biosciences).

As shown in Figure 18A, across each of the splenocyte samples (1 or 2 μ g doses and collected at 11 or 14 days post-boost), mRNA encoding non-MOMP antigens CT443, CT584, CT600 and CT812 gave rise to a CD4+ IFN γ cell response, but which was lower than that elicited by recombinant MOMP protein.

As shown in Figure 18B, across each of the splenocyte samples (1 or 2 μ g doses and collected at 11 or 14 days post-boost), mRNA encoding non-MOMP antigens CT584 and CT812 elicited high CD8+ IFN γ cell responses relative to CT443, CT600 and recombinant MOMP protein.

Non-MOMP Ct antigen constructs are able to induce specific IgG against their encoded proteins

In this example, the following mRNA constructs encoding non-MOMP *Ct* antigens (all modified) were tested for their ability to induce specific IgG against their respective protein: CT443_ssHA1 ("CT443"; encoding protein of SEQ ID NO :105), CT443_ssHA1_GlycNeg ("CT443_GN", encoding protein of SEQ ID NO :106), CT584_ssHA1 ("CT584", encoding protein of SEQ ID NO :107), CT584_ssHA1_Glycneg ("CT584_GN", encoding protein of SEQ ID NO :108), CT584_ssHA1_C2S ("CT584_C2S", encoding protein of SEQ ID NO :109), CT584_ssHA1_Glycneg_C2S ("CT584_GN_C2S", encoding protein of SEQ ID NO :110), CT600_trunc_ssHA1 ("CT600_trunc"; encoding protein of SEQ ID NO: 111), CT600_trunc_ssHA1_Glycneg ("CT600_trunc_GN", encoding protein of SEQ ID NO :112), CT812_pass-domain_ssHA1 ("CT812_pass-dom", encoding protein of SEQ ID NO: 113), CT812_pass-domain_ssHA1_C2S ("CT812_pass-dom_C2S", encoding protein of SEQ ID NO: 114), CT812_ext-pass-domain_ssHA1 ("CT812_ext-pass-dom", encoding protein of SEQ ID NO: 115), CT812_ext-pass-domain_ssHA1_C2S ("CT812_ext-pass-dom_C2S", encoding protein of SEQ ID NO: 116).

Mice (C57Bl/6) received two immunisations of mRNA constructs at 5 μ g dose, formulated with the LNP OF-02, given by IM route, at 0 and 3 weeks (W0 and W3) in at least one of two independent studies. Studies included a negative empty OF-02 LNP control. Blood samples were collected at 0 weeks (W0) and two weeks after the last vaccine administration, in order to measure total IgG by ELISA. The specific IgG were measured from individual sera using automated 384 ELISA.

Briefly, 384-well micro-plates were coated in carbonate buffer with 20 μ L per well of respective protein (CT443 (4 μ g/mL), CT584 (1 μ g/mL), CT600 (4 μ g/mL)) and kept overnight at +4°C. Coating solution was

removed and washed with buffer 1 (PBS/Tween 0.05%). Free sites were blocked with 75 μ L of buffer 2 (PBS / Tween 20 at 0.05% / milk 1%) and incubated 90 min at room temperature (RT). Plates were emptied, then sera were serially diluted in buffer 2 under a volume of 20 μ L (12 times) in the microplates. The plates were incubated for 90 min at RT and then washed with buffer 1. 20 μ L of a diluted anti-mouse total IgG peroxidase conjugate (Southern Biotech) was added in each well (1/1000). After 90 min incubation at RT, the plates were washed with buffer 1. The reaction was developed by adding 20 μ L of a tetramethylbenzidine substrate solution in each well. The reaction was chemically stopped after 30 min at RT with HCl (1N (normality)) and absorbance was measured at 450-650 nm on a spectrophotometer (Synergy HTX, Biotek). The results were analyzed in Softmax Pro software using a standard curve and expressed in arbitrary ELISA units by the reciprocal dilution corresponding to an OD of 1.

As shown in Figure 19, all samples tested gave rise to high titers of IgGs which were specific against their respective protein, with the exception of CT812, which elicited lower IgG titers for all constructs.

mRNA constructs encoding non-MOMP Ct antigens induce a T cell response

Further mRNA constructs (all modified) encoding non-MOMP Ct antigens (CT443_ssHA1 (“CT443”, encoding protein of SEQ ID NO: 105), CT584_ssHA1_GlycNeg (“CT584_GlycNeg”, encoding protein of SEQ ID NO:108), CT600_trunc_ssHA1_GlycNeg (“CT600_GlycNeg”, encoding protein of SEQ ID NO:112) were tested for their ability to induce a T cell response, as compared to a control of mRNA encoding MOMP_serE_FL_ssHA1 (“MOMP ser E FL”; encoding protein of SEQ ID NO: 29).

Mice received two immunisations of mRNA ssHA1-constructs at 0.125 μ g dose, formulated with the LNP OF-02, given by IM route, at 0 and 3 weeks (W0 and W3). Study included a negative empty-LNP (OF-02) and a positive MOMP_serE_FL_ssHA1 control. Splenocytes were collected two weeks post-boost and T specific cellular response was assessed by ELISPOT/FluoroSpot and by ICCS.

Ability to elicit IFN γ -producing T cells (FluoroSpot)

First, ability to elicit IFN γ -producing T cells was tested using FluoroSpot. For the FluoroSpot assay, spleen cells (2×10^5 per well) were plated in 200 μ L RPMI complete together with and without respective native peptide pools (as described above) in presence of IL2 (Roche) in triplicate cultures each. Subsequently plates were incubated at 37°C, 5% CO₂ for 20 h. After washing with PBS, plates were incubated with detection Ab anti-IFN γ (mAb R4-6A2-BAM) for 2 h at RT. Image analysis of FluoroSpot assays was performed on Microvision Reader.

As shown in Figure 20, the CT443 and CT584_GlycNeg constructs were able to induce similarly high, if not enhanced, levels of IFN γ -producing T cells, relative to the MOMP serE FL construct. However, CT600_GlycNeg did not induce similarly high levels of IFN γ -producing T cells.

Ability to elicit CD4+IFN γ +, CD8+IFN γ +, and polyfunctional cell responses (ICCS)

For ICCS, isolated lymphocytes were activated with specific respective peptide pools (as described above) at 1×10^6 cells/well in 96-well plates and cultured for 6 h in 5% CO₂ at 37°C with BFA in order to block cytokine secretion. Activated cells were harvested for surface and intracellular cytokine staining. Surface staining of the cells was performed by incubating with fluorescent conjugated monoclonal antibodies specific to CD3, CD4, and CD8 for 20 min on ice. After surface staining, the cells were washed two times, fixed and permeabilized for 20 min at +4°C using BD Cytofix/Cytoperm buffer (BD Biosciences) and washed twice with the BD Perm/Wash buffer. Intracellular cytokine staining was then performed by incubating with the fluorescent conjugate antibodies against murine IL-2, IL-5, IL-10, TNF- α , and IFN- γ on ice for 30 min. The cells were washed three times and analyzed with a FACS Fortessa flow cytometer (BD Biosciences).

As shown in Figure 21A, the CT443 construct gave rise to a CD4+ IFN γ T cell response, albeit at a lower level relative to MOMP_serE_FL_ssHA1 (“MOMP ser E FL”). Immunisation with either of CT584_GlycNeg or CT600_GlycNeg did not appear to give rise to a CD4+ IFN γ T cell response.

As shown in Figure 21B, the CT443 and CT584_GlycNeg constructs each gave rise to a relatively strong CD8+ IFN γ T cell response. MOMP_serE_FL_ssHA1 (“MOMP ser E FL”) and CT600_GlycNeg CD8+ IFN γ T cell responses were indistinguishable from empty LNP control.

The mRNA constructs were also assessed for their ability to induce polyfunctional cells. Figure 22 shows, for each of the mRNA constructs, the percentages of CD4+ (A) and CD8+ (B) antigen-specific T cells that were elicited and indicates the percentage of CD4+ and CD8+ T cells producing each of IFN γ (“IFN γ ”), IL2, and/or TNF α (“TNF α ”) (singularly and in the various combinations thereof). CD4+ responses were elicited for each of MOMP_serE_FL_ssHA1 (“MOMP ser E FL”) and CT443, and the polyfunctional CD4+IFN γ +IL2+TNF α + cell population formed the majority, relative to the other CD4+ T cell populations (Figure 22A).

CD8+ responses were elicited for each of CT443 and CT584_GlycNeg, and the CD8+IFN γ +IL2-TNF α + cell population formed the majority, relative to the other CD8+ T cell populations (Figure 22B).

Non-MOMP Ct antigen constructs are able to induce specific IgG against their encoded proteins at a low dose of 0.125 μ g

Further non-MOMP Ct antigens were tested for their ability to induce specific IgG against their respective protein. mRNA constructs (all modified) included: CT443_ssHA1 (“CT443”, encoding protein of SEQ ID NO: 105, CT584_ssHA1_GlycNeg (“CT584 mRNA”, encoding protein of SEQ ID NO: 108), CT600_trunc_ssHA1_Glycneg (“CT600 mRNA”, encoding protein of SEQ ID NO: 112). Protein constructs

included: Recombinant (rProt) CT584 (SEQ ID NO: 145), rProt CT600 (SEQ ID NO: 146) and rProt CT443 (SEQ ID NO: 143).

Mice received two immunisations of mRNA constructs and recombinant protein at 0.125 and 5 µg dose respectively, formulated with the LNP OF-02 and SPA14 respectively, given by IM route, at 0 and 3 weeks (W0 and W3). Studies included a negative empty OF-02 LNP control. Blood samples were collected at 0 weeks (W0) and two weeks after the last vaccine administration, in order to measure total IgG by ELISA. The specific IgG were measured from individual sera using automated 384 ELISA.

Briefly, 384-well micro-plates were coated in carbonate buffer with 20 µL per well of respective protein (CT443 (4µg/mL), CT584 (1µg/mL), CT600 (4µg/mL)) and kept overnight at +4°C. Coating solution was removed and washed with buffer 1 (PBS/Tween 0.05%). Free sites were blocked with 75 µL of buffer 2 (PBS / Tween 20 0.05 % / milk 1 %) and incubated 90 min at room temperature (RT). Plates were emptied, then sera were serially diluted in buffer 2 under a volume of 20 µL (12 times) in the microplates. The plates were incubated for 90 min at RT and then washed with buffer 1. 20 µL of a diluted anti-mouse total IgG peroxidase conjugate (Southern Biotech) was added in each well (1/1000). After 90 min incubation at RT, the plates were washed with buffer 1. The reaction was developed by adding 20 µL of a tetramethylbenzidine substrate solution in each well. The reaction was chemically stopped after 30 min at RT with HCl (1N (normality)) and absorbance was measured at 450-650 nm on a spectrophotometer (Synergy HTX, Biotek). The results were analyzed in Softmax Pro software using a standard curve and expressed in arbitrary ELISA units by the reciprocal dilution corresponding to an OD of 1.

As shown in Figure 23, all samples tested gave rise to high titers of IgGs which were specific against their respective protein, even at the low dose of 0.125 µg.

Non-MOMP Ct antigen constructs elicit antibodies binding to elementary bodies (EBs)

The following mRNA constructs of non-MOMP Ct antigens (all modified mRNA) were tested for their ability to induce IgGs binding EBs of serovars E, D, or G. Constructs were formulated with the LNP OF-02. 8 or 10 mice (C57BL/6) were used in each study group and 5 mice were used for each negative control.

The following constructs were tested:

- CT443_ssHA1 ("CT443", encoding protein of SEQ ID NO :105),
- CT443_ssHA1_GlycNeg ("CT443_GN", encoding protein of SEQ ID NO :106),
- CT584_ssHA1 ("CT584", encoding protein of SEQ ID NO :107),
- CT584_ssHA1_Glycneg ("CT584_GN", encoding protein of SEQ ID NO :108),
- CT584_ssHA1_C2S ("CT584_C2S", encoding protein of SEQ ID NO :109),
- CT600_trunc_ssHA1 ("CT600_trunc", encoding protein of SEQ ID NO: 111),

- CT600_trunc_ssHA1_Glycneg (“CT600_trunc_GN”, encoding protein of SEQ ID NO :112) in two independent studies,
- CT812_pass-domain_ssHA1 (“CT812_pass-dom”, encoding protein of SEQ ID NO: 113),
- CT812_pass-domain_ssHA1_C2S (“CT812_pass-dom_CS2”, encoding protein of SEQ ID NO: 114),
- CT812_ext-pass-domain_ssHA1 (“CT812_ext-pass-dom”, encoding protein of SEQ ID NO: 115) in two independent studies,
- CT812_ext-pass-domain_ssHA1_C2S (“CT812_ext-pass-dom_C2S”, encoding protein of SEQ ID NO: 116).

Mice received two immunisations of mRNA constructs at 5 µg dose, formulated with the LNP OF-02, given by IM route, at 0 and 3 weeks (W0 and W3) in at least one of two independent studies. Blood samples were collected at 0 weeks (W0) and two weeks after the last vaccine administration, in order to measure total IgG by ELISA. The specific IgG were measured from individual sera using automated 384 ELISA.

Briefly, 384-well micro-plates were coated in carbonate buffer with 20 µL per well of EBs at 5-15 µg/mL depending on the serovar (5µg/mL for EBs of serovar G, 7,5µg/mL for EBs of serovar D, and 15µg/mL for EBs of serovar E) and kept overnight at +4°C. Coating solution was removed and washed with buffer 1 (PBS/Tween 0.05%). Free sites were blocked with 75 µL of buffer 2 (PBS / Tween 20 0.05 % / milk 1 %) and incubated 90 min at room temperature (RT). Plates were emptied, then sera were serially diluted in buffer 2 under a volume of 20 µL (12 times) in the microplates. The plates were incubated for 90 min at RT and then washed with buffer 1. 20 µL of a diluted anti-mouse total IgG peroxidase conjugate (Jackson Inc) was added in each well (1/2500). After 90 min incubation at RT, the plates were washed with buffer 1. The reaction was developed by adding 20 µL of a tetramethylbenzidine substrate solution in each well. The reaction was chemically stopped after 30 min at RT with HCl (1N (normality)) and absorbance was measured at 450-650 nm on a spectrophotometer (Synergy HTX, Biotek). The results were analyzed in Softmax Pro software using a standard curve and expressed in arbitrary ELISA units by the reciprocal dilution corresponding to an OD of 1.

As shown in Figure 24, various non-MOMP Ct mRNA constructs elicited IgGs which bound to EBs of serovar E. In particular, the CT443, CT584, and CT600 constructs induced acceptable levels of antibodies against EBs of serovar E, which were higher than background levels. Of the CT812 constructs, only the CT812_pass-dom construct induced an acceptable level of antibodies against EBs of serovar E; however, the level remained lower than those observed for the CT443, CT584, and CT600 constructs.

As shown in Figures 25 and 26, CT443 and CT584 constructs also induced acceptable levels of antibodies which bound to EBs of serovars D and G.

were collected at 0 weeks (W0) and two weeks after the last vaccine administration, in order to measure total IgG by ELISA. The specific IgG were measured from individual sera using automated 384 ELISA.

Briefly, 384-well micro-plates were coated in carbonate buffer with 20 μ L per well of recombinant MOMP protein (rMOMP; SEQ ID NO: 134) at 2 μ g/mL and kept overnight at +4°C. Coating solution was removed and washed with buffer 1 (PBS/Tween 0.05%). Free sites were blocked with 75 μ L of buffer 2 (PBS / Tween 20 0.05 % / milk 1 %) and incubated 90 min at room temperature (RT). Plates were emptied, then sera were serially diluted in buffer 2 under a volume of 20 μ L (12 times) in the microplates. The plates were incubated for 90 min at RT and then washed with buffer 1. 20 μ L of a diluted anti-mouse total IgG peroxidase conjugate (Southern Biotech) was added in each well (1/1000). After 90 min incubation at RT, the plates were washed with buffer 1. The reaction was developed by adding 20 μ L of a tetramethylbenzidine substrate solution in each well. The reaction was chemically stopped after 30 min at RT with HCl (1N (normality)) and absorbance was measured at 450-650 nm on a spectrophotometer (Synergy HTX, Biotek). The results were analyzed in Softmax Pro software using a standard curve and expressed in arbitrary ELISA units by the reciprocal dilution corresponding to an OD of 1.

As shown in Figure 27, all samples tested gave rise to high titers of IgGs which were specific against recombinant MOMP, with exception of the MOMP_SerG_FL_ssHA1 construct, which did not give rise to an anti-MOMP IgG response.

EXAMPLE 10 – B cell constructs elicit antibodies binding to elementary bodies (EBs)

B cell constructs (all modified mRNA) were tested for their ability to induce IgGs binding to EBs of serovars E, D, or G. Constructs were formulated with the LNP OF-02, while the recombinant MOMP protein (positive control) was formulated with a liposome-based adjuvant containing TLR4 agonist (SPA14, as described in WO2022090359).

The following constructs were tested:

1. MOMP_VDcomb1-extP_ssHA1 (“P” of Chimera 1, encoding protein of SEQ ID NO: 61)
2. MOMP_VDcomb1-extS_ssHA1 (“S” of Chimera 1, encoding protein of SEQ ID NO: 69)
3. MOMP_VDcomb1-extP_ssHA1_Glycneg (“P_GN” of Chimera 1; encoding protein of SEQ ID NO: 63)
4. MOMP_VDcomb1-extS_ssHA1_Glycneg (“S_GN” of Chimera 1; encoding protein of SEQ ID NO: 71)
5. MOMP_VDcomb1-extP_ssHA1_TMB1_Glycneg (“P_GN_TMB1” of Chimera 1; encoding protein of SEQ ID NO: 67)
6. MOMP_VDcomb1-extS_ssHA1_TMB1_Glycneg (“S_GN_TMB1” of Chimera 1; encoding protein of SEQ ID NO: 75)

7. MOMP_VDcomb2-extP_ssHA1_Glycneg ("P_GN" of Chimera 2, encoding protein of SEQ ID NO: 79)
8. MOMP_VDcomb2-extS_ssHA1_Glycneg ("S_GN" of Chimera 2, encoding protein of SEQ ID NO: 87)
- 5 9. MOMP_VDcomb2-extP_ssHA1_TMB1_Glycneg ("P_GN_TMB1" of Chimera 2, encoding protein of SEQ ID NO: 83)
- 10 10. MOMP_VDcomb2-extS_ssHA1_TMB1 ("S_TMB1" of Chimera 2, encoding protein of SEQ ID NO: 89)
11. MOMP_serD_FL_ssHA1 ("Ser_D", encoding protein of SEQ ID NO: 21)
12. MOMP_serE_FL_ssHA1 ("Ser_E", encoding protein of SEQ ID NO: 29)
13. MOMP_serF_FL_ssHA1 ("Ser_F", encoding protein of SEQ ID NO: 37)
14. MOMP_serG_FL_ssHA1 ("Ser_G", encoding protein of SEQ ID NO: 45)

Mice (C57BL/6) received two immunisations of mRNA constructs at 5 µg dose, formulated with the LNP OF-02, given by IM route, at 0 and 3 weeks (W0 and W3) in at least one of two independent studies. Studies included a negative empty-LNP control and a positive full-length recombinant MOMP protein control (rMOMP; SEQ ID NO: 134, indicated as 'rec Ser E' in Figures 28-30). Blood samples were collected at 0 weeks (W0) and two weeks after the last vaccine administration, in order to measure total IgG by ELISA. The specific IgG were measured from individual sera using automated 384 ELISA.

Briefly, 384-well micro-plates were coated in carbonate buffer with 20 µL per well of EBs at 5-15 µg/mL per serovar (5µg for EBs of serovar G, 7,5µg for EBs of serovar D, and 15µg for EBs of serovar E) and kept overnight at +4°C. Coating solution was removed and washed with buffer 1 (PBS/Tween 0.05%). Free sites were blocked with 75 µL of buffer 2 (PBS / Tween 20 0.05 % / milk 1 %) and incubated 90 min at room temperature (RT). Plates were emptied, then sera were serially diluted in buffer 2 under a volume of 20 µL (12 times) in the microplates. The plates were incubated for 90 min at RT and then washed with buffer 1. 20 µL of a diluted anti-mouse total IgG peroxidase conjugate (Jackson Inc) was added in each well (1/2500). After 90 min incubation at RT, the plates were washed with buffer 1. The reaction was developed by adding 20 µL of a tetramethylbenzidine substrate solution in each well. The reaction was chemically stopped after 30 min at RT with HCl (1N (normality)) and absorbance was measured at 450-650 nm on a spectrophotometer (Synergy HTX, Biotek). The results were analyzed in Softmax Pro software using a standard curve and expressed in arbitrary ELISA units by the reciprocal dilution corresponding to an OD of 1.

As shown in Figure 28, various MOMP B cell constructs elicited high titers of IgGs which bound to EBs of serovar E at levels similar to the recombinant MOMP. mRNA constructs comprising a transmembrane domain, as well as mRNA coding full-length MOMP, did not give rise to an anti-EB IgG response, as titers were similar to that of the empty LNP control.

As shown in Figures 29 and 30, similar results were observed with binding to EBs of serovar D and serovar G.

EXAMPLE 11 – Immunisation with MOMP mRNA elicits a similar immune response with different LNP formulations

5 Mice (female C57BL/6) were immunised with mRNA (all modified) encoding recombinant MOMP protein formulated in either of two LNPs. The following groups were evaluated:

- MOMP P3 ssHA1 (“P3”, encoding protein of SEQ ID NO: 53) at a 2 µg dose, formulated in LNP GL-HEPES-E3-E12-DS-4-E10 (“Lipid D”) or LNP OF-02 (“Lipid A”),
- MOMP_VDcomb2-extS_ssHA1_Glycneg, (“C2-S-GN”, encoding protein of SEQ ID NO: 87) at a
10 2 µg or 5 µg dose, formulated in LNP GL-HEPES-E3-E12-DS-4-E10 (“Lipid D”) or LNP OF-02 (“Lipid A”).

Mice received two immunisations of mRNA constructs by IM route, at 0 and 3 weeks (W0 and W3). W3 is alternatively referred to as day 21 herein. The study included empty LNP controls. Blood samples were collected at 0 weeks (W0) and final blood samples and spleens were collected one or two weeks after the
15 last vaccine administration. The cellular response was evaluated in samples collected one week post vaccination with a 2 µg dose and the humoral response was evaluated in samples collected two weeks post vaccination with a 5 µg dose. The T specific cellular response was assessed by Intra cellular cell staining (ICCS), as described in Example 7. Induction of specific IgG against recombinant MOMP protein and IgGs binding to EBs of serovars E was assessed as described in Example 8.

20 ***MOMP T cell construct elicits a strong T cell response when formulated in different LNPs***

Immunisation with MOMP_P3_ssHA1 (“P3”) gave rise to a significant CD4+ IFNγ T cell response, but very low to no CD8+ IFNγ T cell response was observed, as shown in Figures 31A and 31B, respectively. The level of the response was similar between the two formulations evaluated with P3. In contrast, immunisation with the B cell construct (“C2-S-GN”) gave rise to both a CD4+ IFNγ T cell response and a CD8+ IFNγ T
25 cell response.

Constructs were also assessed for their ability to induce polyfunctional cells. Figure 31C shows that the CD4+IFNγ+IL2+TNFα+ cell population was induced by P3 in both formulations, at similar levels, as well as by the B cell construct.

30 ***MOMP B cell construct induces specific IgG against rMOMP and against EBs of serovar E when formulated in different LNPs***

The MOMP B cell construct (“C2-S-GN”) gave rise to high titers of IgGs which were specific against recombinant MOMP of serovar E (Figure 32A) and EBs of serovar E (Figure 32B) when formulated in different LNPs. Similar levels of IgG were observed between the two formulations.

EXAMPLE 12 – Immunisation with a multivalent mRNA composition elicits an immune response

Mice (female C57BL/6) were immunised with a multivalent composition composed of four mRNAs (all modified) encoding the following recombinant proteins:

- MOMP P3 ssHA1, (“P3”, encoding protein of SEQ ID NO: 53),
- 5 - MOMP_VDcomb2-extS_ssHA1_Glycneg, (“C2-S-GN”, encoding protein of SEQ ID NO: 87),
- CT443_ssHA1 (“CT443”, encoding protein of SEQ ID NO: 105), and
- CT584_ssHA1_GlycNeg (“CT584”, encoding protein of SEQ ID NO: 108).

The ratio of the mRNAs was 1:1:1:1. Three doses were evaluated. Specifically, doses of 0.4 µg, 1.2 µg and 3.6 µg of each mRNA were evaluated, corresponding to a total mRNA dose of 1.6 µg, 4.8µg, and 14.4 µg, respectively for each immunisation. The mRNA composition was formulated in LNP GL-HEPES-E3-E12-DS-4-E10 (“Lipid D”) or LNP IM-001 (“Lipid G”). The study included empty LNPs equivalent to the amount present for the two highest mRNA doses, as controls. The immunisation and sampling protocols were as described in Example 11. The T specific cellular response was assessed by Intra cellular cell staining (ICCS), as described in Example 7. Evaluation of the induction of specific IgG against recombinant MOMP protein and IgGs binding to EBs of serovars E was performed as described in Example 8. Little to

Multivalent composition elicits CD4+IFN γ +, CD8+IFN γ +, and polyfunctional cell responses (ICCS) when formulated in different LNPs

The multivalent composition was tested for its ability to elicit MOMP-specific CD4+ IFN γ + cells, CD8+ IFN γ + T cells, and polyfunctional cells in mice. As shown in Figure 33A, the composition gave rise to a significant CD4+ IFN γ T cell response that was distinguishable from the empty LNP control. For the multivalent composition, the CD4+ IFN γ +IL2+TNF α + cell population formed the majority, relative to the other cell populations (see Figure 33B). Little to no IL-17 or IL-10 was observed for any of the tested constructs (data not shown).

The multivalent composition elicited low levels CD8+ IFN γ (see Figure 34A), with the CD8+ IFN γ +IL2+TNF α + cell population forming the majority, relative to the other cell populations (see Figure 34B). No difference in the response could be observed between the various doses of mRNA formulated in “Lipid D”. A difference between the highest dose and the two lower doses was observed for the mRNA formulated in “Lipid G”.

Multivalent composition induces specific IgG against rMOMP and against EBs of serovar E when formulated in different LNPs

As shown in Figure 35A, the multivalent composition gave rise to high titers of IgGs which were specific against recombinant MOMP of serovar E at all doses and with both formulations. A dose effect was observed.

The multivalent composition also gave rise to high titers of specific IgGs which bound to EBs of serovar E at all doses and with both formulations, as shown in Figure 35B.

Non-MOMP Ct antigen constructs present in the multivalent composition are able to induce specific IgG against their encoded proteins

CT443 and CT584 constructs present in the multivalent composition induce high levels of specific IgG against recombinant CT443 and CT584, respectively, as shown in panels A (CT443) and B (CT584) of Figure 36. Results were similar between the two formulations tested. Results suggest that interference among antigens did not occur, as high levels of specific IgG were induced against each recombinant protein.

EXAMPLE 13 – Immunisation with a multivalent mRNA composition elicits antibody binding to elementary bodies (EBs) of serovar E and serovar G and respective recombinant proteins

Mice (female C57BL/6) were immunised with monovalent or multivalent mRNAs (all modified) encoding the following recombinant proteins:

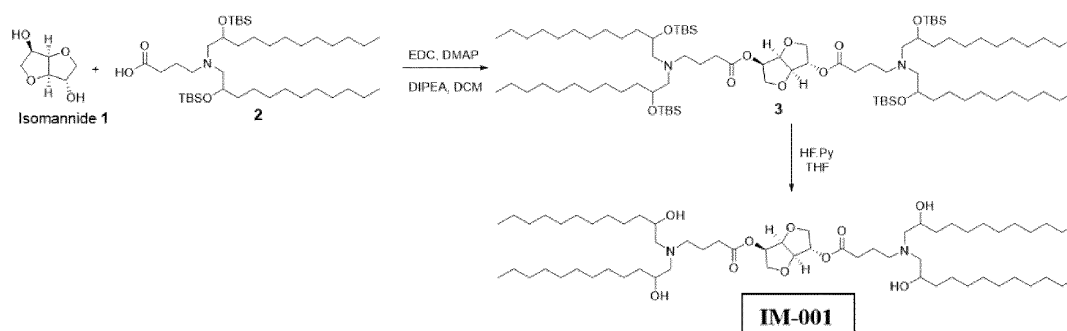
- MOMP P3 ssHA1, ("P3", encoding protein of SEQ ID NO: 53),
- MOMP_VDcomb2-extS_ssHA1_Glycneg, ("C2-S-GN", encoding protein of SEQ ID NO: 87),
- CT443_ssHA1 ("CT443", encoding protein of SEQ ID NO: 105), and
- CT584_ssHA1_GlycNeg ("CT584", encoding protein of SEQ ID NO: 108),
- a multivalent composition including the mRNAs encoding MOMP P3, MOMP C2-S-GN, CT443 and CT584-GN described above.

Mice received two immunisations of mRNA constructs at 1.2 µg dose, formulated in LNP GL-HEPES-E3-E12-DS-4-E10 ("Lipid D") or LNP IM-001 ("Lipid G"), given by IM route, at 0 and 3 weeks (W0 and W3). The multivalent was formulated with a ratio of 1:1:1:1 of each mRNA construct and injected at 1.2 µg dose of each mRNA construct, 4.8 µg of total mRNA. PBS buffer was administered as a control. Blood samples were collected at 0 weeks (W0) and one week after the last vaccine administration to determine seroconversion, which can be detected at this time point. Seroconversion was determined by measuring total IgG from individual sera using automated 384 ELISA as described above in Example 8 for non-MOMP antigen constructs and Example 9 for MOMP antigen constructs. IgG titers were analyzed using one-way ANOVA with group as fixed factor. A Dunnett adjustment was performed.

As shown in Figures 37 and 38, all monovalent and multivalent constructs elicited moderate to high titers of IgGs which were specific against EBs of serovar E and G except the monovalent MOMP_P3. MOMP_P3 mRNA construct did not give rise to an anti-EB IgG response, as titers were similar to that of the buffer control. Similarly, as shown in Figure 39, all monovalent and multivalent constructs elicited moderate to high titers of IgGs which were specific against their respective recombinant protein except the monovalent MOMP P3.

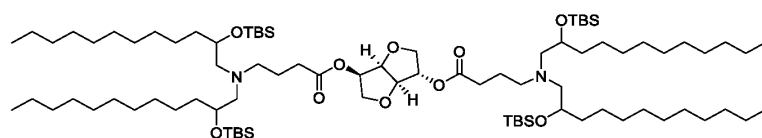
EXAMPLE 14 – Synthesis of IM-001 according to Scheme 2

Scheme 2: General Synthetic Scheme for Lipid of Formula (XI)



Abbreviations: DCM: Dichloromethane, DIPEA: N,N-Diisopropylethylamine, DMAP: 4-Dimethylaminopyridine, EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, EtOAc: Ethyl acetate, NaHCO₃: Sodium hydrogencarbonate, Py: Pyridine, Na₂SO₄: Sodium Sulfate, TEA: Triethylamine, TFA: Trifluoroacetic Acid, MS: Mass spectrometry, ESI-MS: Electrospray ionization mass spectrometry, TLC: Thin Layer Chromatography

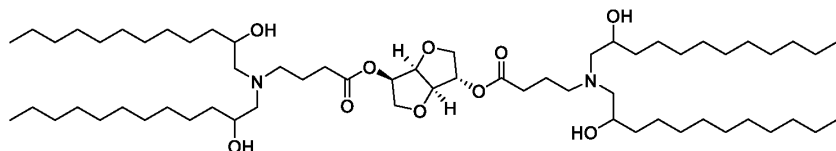
Step 1: Synthesis of Intermediate (3)



As depicted in Scheme 2: To a solution of acid (2) (1.2 g, 1.71 mmol) and isosorbide (1) (0.100 g, 0.68 mmol) in dichloromethane (10 mL) were added DIPEA (0.95 mL, 5.47 mmol), DMAP (0.084 g, 0.68 mmol) and EDC (0.393 g, 2.05 mmol). The resulting mixture was stirred at room temperature for overnight. After 16 h, MS and TLC (30% EtOAc in hexanes) analysis indicated completion of the reaction. The reaction mixture was diluted with dichloromethane and washed with saturated NaHCO₃ solution, water and brine solution. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude residue was purified, and the desired product was eluted at 6% EtOAc in hexanes. The product containing fractions were concentrated to obtain 0.72 g (69%) of pure product.

Results:

ESI-MS: Calculated $C_{86}H_{177}N_2O_{10}Si_4$, $[M + H^+] = 1510.25$, Observed = 1510.3 and 755.4 $[M/2 + H^+]$

Step 2: Synthesis of IM-001

- 5 As depicted in Scheme 2: To a solution of Intermediate (3) (0.72 g, 0.476 mmol) in tetrahydrofuran (4 mL) was added hydrogen fluoride (70% HF.py complex, 2 mL, 14.298 mmol) at 0 °C and stirred at the same temperature for 5 minutes. Then reaction mixture was warmed to room temperature and stirred for 16 h. MS analysis indicated completion of the reaction. The reaction mixture was diluted with ethyl acetate, quenched by slow addition of solid $NaHCO_3$ at 0 °C, followed by saturated $NaHCO_3$ solution. The organic layer was washed with sat. $NaHCO_3$ solution, water and brine. Then dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified, and the desired product was eluted at 65% EtOAc in hexanes. The purest fractions were concentrated to obtain 0.120 g (24%) of pure product.
- 10

Results:

- 1H NMR (400 MHz, $CDCl_3$) δ 5.30 – 5.00 (m, 2H), 4.97 – 4.68 (m, 2H), 4.55 – 3.71 (m, 8H), 3.57 – 2.92 (m, 8H), 2.84 – 2.04 (m, 8H), 1.99 – 1.01 (m, 76H), 0.88 (t, $J = 6.8$ Hz, 12H).
- 15

ESI-MS: Calculated $C_{62}H_{121}N_2O_{10}$, $[M + H^+] = 1053.90$, Observed = 1053.2 and 527.3 $[M/2 + H^+]$

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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SEQUENCES

Table 10 - Protein sequences

(*) for protein sequences where two corresponding nucleic acid sequences are mentioned, the first sequence was tested in mice (where applicable)

SEQ ID NO	name	nt SEQ ID NO(*)	other name
	Full Length constructs		
21	MOMP_serD_FL_ssHA1	879 294	
22	MOMP_serD_FL_ssHA2	301 302	
23	MOMP_serD_FL_ssHA1_C2S	295 296	
24	MOMP_serD_FL_ssHA2_C2S	303 304	
25	MOMP_serD_FL_ssHA1_Glycneg	298 297	
26	MOMP_serD_FL_ssHA2_Glycneg	305 306	
27	MOMP_serD_FL_ssHA1_C2S_Glycneg	873 300	
28	MOMP_serD_FL_ssHA2_C2S_Glycneg	307 308	
29	MOMP_serE_FL_ssHA1	875 309	
30	MOMP_serE_FL_ssHA2	317 318	
31	MOMP_serE_FL_ssHA1_C2S	311 312	
32	MOMP_serE_FL_ssHA2_C2S	319 320	
33	MOMP_serE_FL_ssHA1_Glycneg	314 313	
34	MOMP_serE_FL_ssHA2_Glycneg	321 322	
35	MOMP_serE_FL_ssHA1_C2S_Glycneg	876 316	
36	MOMP_serE_FL_ssHA2_C2S_Glycneg	323 324	
37	MOMP_serF_FL_ssHA1	880 326	
38	MOMP_serF_FL_ssHA2	333 334	
39	MOMP_serF_FL_ssHA1_C2S	327 328	
40	MOMP_serF_FL_ssHA2_C2S	335 336	
41	MOMP_serF_FL_ssHA1_Glycneg	329 330	
42	MOMP_serF_FL_ssHA2_Glycneg	338 337	
43	MOMP_serF_FL_ssHA1_C2S_Glycneg	331 332	
44	MOMP_serF_FL_ssHA2_C2S_Glycneg	339 340	
45	MOMP_serG_FL_ssHA1	881 342	
46	MOMP_serG_FL_ssHA2	349 350	
47	MOMP_serG_FL_ssHA1_C2S	343 874	
48	MOMP_serG_FL_ssHA2_C2S	351 352	
49	MOMP_serG_FL_ssHA1_Glycneg	345 346	
50	MOMP_serG_FL_ssHA2_Glycneg	353 354	
51	MOMP_serG_FL_ssHA1_C2S_Glycneg	347 348	
52	MOMP_serG_FL_ssHA2_C2S_Glycneg	355 356	
	T cell constructs		
53	MOMP_P3_ssHA1	213 214	
54	MOMP_P3_ssHA2	215 216	
55	MOMP_P3_ssHA1_C2S	218 217	
56	MOMP_P3_ssHA2_C2S	219 220	
57	MOMP_P5_ssHA1	222 221	
58	MOMP_P5_ssHA2	223 224	
59	MOMP_P5_ssHA1_C2S	225 226	
60	MOMP_P5_ssHA2_C2S	227 228	

	VD constructs		
61	MOMP_VDcomb1-extP_ssHA1	863 229	
62	MOMP_VDcomb1-extP_ssHA2	237 238	
63	MOMP_VDcomb1-extP_ssHA1_Glycneg	877 232	
64	MOMP_VDcomb1-extP_ssHA2_Glycneg	239 240	
65	MOMP_VDcomb1-extP_ssHA1_TMB1	233 234	
66	MOMP_VDcomb1-extP_ssHA2_TMB2	241 242	
67	MOMP_VDcomb1-extP_ssHA1_TMB1_Glycneg	865 235	
68	MOMP_VDcomb1-extP_ssHA2_TMB2_Glycneg	243 244	
69	MOMP_VDcomb1-extS_ssHA1	864 245	
70	MOMP_VDcomb1-extS_ssHA2	253 254	
71	MOMP_VDcomb1-extS_ssHA1_Glycneg	878 248	
72	MOMP_VDcomb1-extS_ssHA2_Glycneg	255 256	
73	MOMP_VDcomb1-extS_ssHA1_TMB1	249 250	
74	MOMP_VDcomb1-extS_ssHA2_TMB2	257 258	
75	MOMP_VDcomb1-extS_ssHA1_TMB1_Glycneg	866 251	
76	MOMP_VDcomb1-extS_ssHA2_TMB2_Glycneg	259 260	
77	MOMP_VDcomb2-extP_ssHA1	261 262	
78	MOMP_VDcomb2-extP_ssHA2	269 270	
79	MOMP_VDcomb2-extP_ssHA1_Glycneg	868 264	
80	MOMP_VDcomb2-extP_ssHA2_Glycneg	869 272	
81	MOMP_VDcomb2-extP_ssHA1_TMB1	265 266	
82	MOMP_VDcomb2-extP_ssHA2_TMB2	273 274	
83	MOMP_VDcomb2-extP_ssHA1_TMB1_Glycneg	871 268	
84	MOMP_VDcomb2-extP_ssHA2_TMB2_Glycneg	867 276	
85	MOMP_VDcomb2-extS_ssHA1	277 278	
86	MOMP_VDcomb2-extS_ssHA2	285 286	
87	MOMP_VDcomb2-extS_ssHA1_Glycneg	870 280	
88	MOMP_VDcomb2-extS_ssHA2_Glycneg	287 288	
89	MOMP_VDcomb2-extS_ssHA1_TMB1	872 282	
90	MOMP_VDcomb2-extS_ssHA2_TMB2	289 290	
91	MOMP_VDcomb2-extS_ssHA1_TMB1_Glycneg	283 284	
92	MOMP_VDcomb2-extS_ssHA2_TMB2_Glycneg	291 292	
	BENCHMARK constructs		
93	Hirep2_ssHA1	862 202	
94	Hirep2_ssHA1_TMB1	203 204	
95	Hirep2_ssHA1_Glycneg	205 206	
96	Hirep2_ssHA1_TMB1_Glycneg	207 208	
97	CTH522_ssHA1	209 210	
98	CTH522_ssHA1_Glycneg	211 212	
99	Hirep2_patent_ssHA1	357 358	
100	Hirep2_patent_ssHA1_TMB1	359 360	
101	Hirep2_patent_ssHA1_Glycneg	361 362	
102	Hirep2_patent_ssHA1_TMB1_Glycneg	363 364	
103	CTH522_patent_ssHA1	366 365	
104	CTH522_patent_ssHA1_Glycneg	367 368	
	CT443		
105	CT443_ssHA1	369 370	
106	CT443_ssHA1_Glycneg	371 372	
	CT584		
107	CT584_ssHA1	374 373	
108	CT584_ssHA1_Glycneg	377 378	
109	CT584_ssHA1_C2S	376 375	

110	CT584_ssHA1_Glycneg_C2S	379 380	
	CT600		
111	CT600_trunc_ssHA1	381 382	
112	CT600_trunc_ssHA1_Glycneg	383 384	
	CT812		
113	CT812_pass-domain_ssHA1	385 386	
114	CT812_pass-domain_ssHA1_C2S	388 387	
115	CT812_ext-pass-domain_ssHA1	389 390	
116	CT812_ext-pass-domain_ssHA1_C2S	391 392	
117	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1	399 400	
118	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3	411 412	
119	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1	393 394	
120	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3	405 406	
121	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1_C2S	413 414	
122	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3_C2S	417 418	
123	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1_C2S	395 396	
124	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3_C2S	407 408	
125	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1	403 404	
126	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3	401 402	
127	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1	421 422	
128	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3	419 420	
129	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1_C2S	423 424	
130	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3_C2S	415 416	
131	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1_C2S	409 410	
132	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3_C2S	397 398	
	T cell MOMP protein		
133	MOMP_P5_6his_coli		
	Full Length MOMP protein		
134	MOMP_SerE_FL_Wt		
135	MOMP_serE_FL_6his_coli		
136	MOMP_serE_FL_C2S_6his_coli		
	BENCHMARK constructs protein		
142	rec SSI CTH522_6his_coli		
	Non-MOMP constructs		
143	CT443_6his_coli	538	
144	CT443NterTrunc_6his_coli		
145	6his_CT584_coli	536	
146	6his_CT600_coli	537	
147	CT812_ext-pass-domain_6his_coli		
197	rCT812		
198	rCT443		
	Pilot constructs		
148	MC3 MOMP or MC3 SER E MOMP	425	OmpA Serovar E
149	MC3 MOMP (human leader) or MC3 SER E MOMP human leader	426	OmpA Serovar E w/ Human Leader
150	OmpA Serovar D w/ Human Leader		
151	OF-02 MOMP or OF-02 SER E MOMP	425	OmpA Serovar E
152	OF-02 MOMP (human leader) or OF-02 SER E MOMP human leader	426	OmpA Serovar E w/ Human Leader

153	-	427	OmpA Serovar D w/ Human Leader
154	mRNA MOMP WT	425	OmpA Serovar E
155	mRNA MNR MOMP E FL MRT12514	861	MNR CT-681 (wild type)-no tag
156	mRNA MOMP Δ VD4	428	MOMP/D-VD4
157	mRNA MOMP Δ VD24 or mRNA UNR MOMPE DII:IV	429	MOMP/D-VD2_VD4
158	mRNA MOMP Δ VD24	430	MOMP/D-VD1_VD2_VD4
159	mRNA MOMP Δ VD1234	431	MOMP/ D-VD3_VD1_VD2_VD4
160	mRNA MOMP Δ signal peptide	432	MOMP/ D-signalpep
161	mRNA MOMP Δ VD124	452	MOMP:DVD1
162	mRNA MOMP Δ VD2	453	MOMP:DVD2
163	mRNA MOMP Δ VD3	454	MOMP:DVD3
164	mRNA MOMP Δ VD23	455	MOMP:DVD2:DVD3
165	mRNA MOMP Δ VD123	456	MOMP:DVD1:DVD2:DVD3
166	mRNA MOMP Δ VD234	457	MOMP:DVD2:DVD3:DVD4
167	mRNA MOMP Δ VD134	458	MOMP:DVD1:DVD3:DVD4
168	CT812: 1-1530	433	CT812:1-1530
169	CT812: 1-761	434	CT812:1-761
170	Nan96-SS:CT812: 32-761 or CT812	435	Nan96-SS:CT812:32-761
171	CT600: 1-188	436	CT600:1-188
172	Nan96-SS:CT600: 2-188 or CT600	437	Nan96HA-SS:CT600:2-188
173	CT443: 1-576	438	CT443:1-576
174	Nan96-SS:CT443: 32-576 or CT443	439	Nan96HA-SS:CT443:32-576
175	CT584:1-183	440	CT584:1-183
176	CT584:1-183*	441	CT584:1-183*
177	Nan96HA-SS:CT584:2-183 or CT584	442	Nan96HA-SS:CT584:2-183
	BENCHMARK constructs		
506	Hirep2		
841	Hirep2_patent	695 696	
842	CTH522		
843	CTH522_patent		

Table 11 - Sequence information

SEQ ID NO:	Name	SEQ ID NO:	Name
	P3 and P5 VD loops	499	MOMP_VDcomb2-extP_Glycneg

462	VD1_P3	500	MOMP_VDcomb2-extP_TMB1
463	VD2_P3	501	MOMP_VDcomb2-extP_TMB1_Glycneg
464	VD3_P3	502	MOMP_VDcomb2-extS
465	VD4_P3	503	MOMP_VDcomb2-extS_Glycneg
466	VD1_P5	504	MOMP_VDcomb2-extS_TMB1
467	VD2_P5	505	MOMP_VDcomb2-extS_TMB1_Glycneg
468	VD3_P5		non-MOMP proteins without SS
469	VD4_P5	507	CT443
	MOMP proteins without SS	508	CT443_Glycneg
470	MOMP_serD_FL		CT584
471	MOMP_serD_FL_C2S	509	CT584
472	MOMP_serD_FL_Glycneg	510	CT584_Glycneg
473	MOMP_serD_FL_C2S_Glycneg	511	CT584_C2S
474	MOMP_serE_FL	512	CT584_Glycneg_C2S
475	MOMP_serE_FL_C2S		CT600
476	MOMP_serE_FL_Glycneg	844	CT600_ref
477	MOMP_serE_FL_C2S_Glycneg	513	CT600_trunc
478	MOMP_serF_FL	514	CT600_trunc_Glycneg
479	MOMP_serF_FL_C2S		CT812
480	MOMP_serF_FL_Glycneg	515	CT812_ref
481	MOMP_serF_FL_C2S_Glycneg	516	CT812_pass-domain
482	MOMP_serG_FL	517	CT812_pass-domain_C2S
483	MOMP_serG_FL_C2S	518	CT812_ext-pass-domain
484	MOMP_serG_FL_Glycneg	519	CT812_ext-pass-domain_C2S
485	MOMP_serG_FL_C2S_Glycneg	520	CT812_pass-domain_HPX1-1_HPX2-1
	T cell constructs	521	CT812_pass-domain_HPX1-1_HPX2-3
486	MOMP_P3	522	CT812_pass-domain_HPX1-2_HPX2-1
487	MOMP_P3_C2S	523	CT812_pass-domain_HPX1-2_HPX2-3
488	MOMP_P5	524	CT812_pass-domain__HPX1-1_HPX2-1_C2S
489	MOMP_P5_C2S	525	CT812_pass-domain_HPX1-1_HPX2-3_C2S

	VD constructs		
490	MOMP_VDcomb1-extP	526	CT812_pass-domain_HPX1-2_HPX2-1_C2S
491	MOMP_VDcomb1-extP_Glycneg	527	CT812_pass-domain_HPX1-2_HPX2-3_C2S
492	MOMP_VDcomb1-extP_TMB1	528	CT812_ext-pass-domain_HPX1-1_HPX2-1
493	MOMP_VDcomb1-extP_TMB1_Glycneg	529	CT812_ext-pass-domain_HPX1-1_HPX2-3
494	MOMP_VDcomb1-extS	530	CT812_ext-pass-domain_HPX1-2_HPX2-1
495	MOMP_VDcomb1-extS_Glycneg	531	CT812_ext-pass-domain_HPX1-2_HPX2-3
496	MOMP_VDcomb1-extS_TMB1	532	CT812_ext-pass-domain_HPX1-1_HPX2-1_C2S
		533	CT812_ext-pass-domain_HPX1-1_HPX2-3_C2S

Table 12 - Nucleic acid sequences

SEQ ID NO:	Construct name	Corresp. SEQ ID NO. (with SS)
539	Hirep2_ssHA1	862
540	Hirep2_ssHA1	202
541	Hirep2_ssHA1_TMB1	203
542	Hirep2_ssHA1_TMB1	204
543	Hirep2_ssHA1_Glycneg	205
544	Hirep2_ssHA1_Glycneg	206
545	Hirep2_ssHA1_TMB1_Glycneg	207
546	Hirep2_ssHA1_TMB1_Glycneg	208
547	CTH522_ssHA1	209
548	CTH522_ssHA1	210
549	CTH522_ssHA1_Glycneg	211
550	CTH522_ssHA1_Glycneg	212
551	MOMP_P3_ssHA1	213
552	MOMP_P3_ssHA1	214
553	MOMP_P3_ssHA2	215
554	MOMP_P3_ssHA2	216
555	MOMP_P3_ssHA1_C2S	217
556	MOMP_P3_ssHA1_C2S	218
557	MOMP_P3_ssHA2_C2S	219
558	MOMP_P3_ssHA2_C2S	220
559	MOMP_P5_ssHA1	221
560	MOMP_P5_ssHA1	222
561	MOMP_P5_ssHA2	223

562	MOMP_P5_ssHA2	224
563	MOMP_P5_ssHA1_C2S	225
564	MOMP_P5_ssHA1_C2S	226
565	MOMP_P5_ssHA2_C2S	227
566	MOMP_P5_ssHA2_C2S	228
567	MOMP_VDcomb1-extP_ssHA1	229
568	MOMP_VDcomb1-extP_ssHA1	863
569	MOMP_VDcomb1-extP_ssHA1_Glycneg	877
570	MOMP_VDcomb1-extP_ssHA1_Glycneg	232
571	MOMP_VDcomb1-extP_ssHA1_TMB1	233
572	MOMP_VDcomb1-extP_ssHA1_TMB1	234
573	MOMP_VDcomb1-extP_ssHA1_TMB1_Glycneg	235
574	MOMP_VDcomb1-extP_ssHA1_TMB1_Glycneg	865
575	MOMP_VDcomb1-extP_ssHA2	237
576	MOMP_VDcomb1-extP_ssHA2	238
577	MOMP_VDcomb1-extP_ssHA2_Glycneg	239
578	MOMP_VDcomb1-extP_ssHA2_Glycneg	240
579	MOMP_VDcomb1-extP_ssHA2_TMB2	241
580	MOMP_VDcomb1-extP_ssHA2_TMB2	242
581	MOMP_VDcomb1-extP_ssHA2_TMB2_Glycneg	243
582	MOMP_VDcomb1-extP_ssHA2_TMB2_Glycneg	244
583	MOMP_VD-comb1-extS_ssHA1	245
584	MOMP_VD-comb1-extS_ssHA1	864
585	MOMP_VD-comb1-extS_ssHA1_Glycneg	878
586	MOMP_VD-comb1-extS_ssHA1_Glycneg	248
587	MOMP_VD-comb1-extS_ssHA1_TMB1	249
588	MOMP_VD-comb1-extS_ssHA1_TMB1	250
589	MOMP_VD-comb1-extS_ssHA1_TMB1_Glycneg	251
590	MOMP_VD-comb1-extS_ssHA1_TMB1_Glycneg	866
591	MOMP_VD-comb1-extS_ssHA2	253
592	MOMP_VD-comb1-extS_ssHA2	254
593	MOMP_VD-comb1-extS_ssHA2_Glycneg	255
594	MOMP_VD-comb1-extS_ssHA2_Glycneg	256
595	MOMP_VD-comb1-extS_ssHA2_TMB2	257
596	MOMP_VD-comb1-extS_ssHA2_TMB2	258
597	MOMP_VD-comb1-extS_ssHA2_TMB2_Glycneg	259
598	MOMP_VD-comb1-extS_ssHA2_TMB2_Glycneg	260
599	MOMP_VDcomb2-extP_ssHA1	261
600	MOMP_VDcomb2-extP_ssHA1	262
601	MOMP_VDcomb2-extP_ssHA1_Glycneg	868
602	MOMP_VDcomb2-extP_ssHA1_Glycneg	264
603	MOMP_VDcomb2-extP_ssHA1_TMB1	265
604	MOMP_VDcomb2-extP_ssHA1_TMB1	266
605	MOMP_VDcomb2-extP_ssHA1_TMB1_Glycneg	871
606	MOMP_VDcomb2-extP_ssHA1_TMB1_Glycneg	268
607	MOMP_VDcomb2-extP_ssHA2	269
608	MOMP_VDcomb2-extP_ssHA2	270
609	MOMP_VDcomb2-extP_ssHA2_Glycneg	869
610	MOMP_VDcomb2-extP_ssHA2_Glycneg	272
611	MOMP_VDcomb2-extP_ssHA2_TMB2	273
612	MOMP_VDcomb2-extP_ssHA2_TMB2	274
613	MOMP_VDcomb2-extP_ssHA2_TMB2_Glycneg	867
614	MOMP_VDcomb2-extP_ssHA2_TMB2_Glycneg	276

615	MOMP_VDcomb2-extS_ssHA1	277
616	MOMP_VDcomb2-extS_ssHA1	278
617	MOMP_VDcomb2-extS_ssHA1_Glycneg	870
618	MOMP_VDcomb2-extS_ssHA1_Glycneg	280
619	MOMP_VDcomb2-extS_ssHA1_TMB1	872
620	MOMP_VDcomb2-extS_ssHA1_TMB1	282
621	MOMP_VDcomb2-extS_ssHA1_TMB1_Glycneg	283
622	MOMP_VDcomb2-extS_ssHA1_TMB1_Glycneg	284
623	MOMP_VDcomb2-extS_ssHA2	285
624	MOMP_VDcomb2-extS_ssHA2	286
625	MOMP_VDcomb2-extS_ssHA2_Glycneg	287
626	MOMP_VDcomb2-extS_ssHA2_Glycneg	288
627	MOMP_VDcomb2-extS_ssHA2_TMB2	289
628	MOMP_VDcomb2-extS_ssHA2_TMB2	290
629	MOMP_VDcomb2-extS_ssHA2_TMB2_Glycneg	291
630	MOMP_VDcomb2-extS_ssHA2_TMB2_Glycneg	292
631	MOMP_serD_FL_ssHA1	879
632	MOMP_serD_FL_ssHA1	294
633	MOMP_serD_FL_ssHA1_C2S	295
634	MOMP_serD_FL_ssHA1_C2S	296
635	MOMP_serD_FL_ssHA1_Glycneg	297
636	MOMP_serD_FL_ssHA1_Glycneg	298
637	MOMP_serD_FL_ssHA1_C2S_Glycneg	873
638	MOMP_serD_FL_ssHA1_C2S_Glycneg	300
639	MOMP_serD_FL_ssHA2	301
640	MOMP_serD_FL_ssHA2	302
641	MOMP_serD_FL_ssHA2_C2S	303
642	MOMP_serD_FL_ssHA2_C2S	304
643	MOMP_serD_FL_ssHA2_Glycneg	305
644	MOMP_serD_FL_ssHA2_Glycneg	306
645	MOMP_serD_FL_ssHA2_C2S_Glycneg	307
646	MOMP_serD_FL_ssHA2_C2S_Glycneg	308
647	MOMP_serE_FL_ssHA1	309
648	MOMP_serE_FL_ssHA1	875
649	MOMP_serE_FL_ssHA1_C2S	311
650	MOMP_serE_FL_ssHA1_C2S	312
651	MOMP_serE_ssHA1_Glycneg	313
652	MOMP_serE_ssHA1_Glycneg	314
653	MOMP_serE_ssHA1_C2S_Glycneg	876
654	MOMP_serE_ssHA1_C2S_Glycneg	316
655	MOMP_serE_FL_ssHA2	317
656	MOMP_serE_FL_ssHA2	318
657	MOMP_serE_FL_ssHA2_C2S	319
658	MOMP_serE_FL_ssHA2_C2S	320
659	MOMP_serE_ssHA2_Glycneg	321
660	MOMP_serE_ssHA2_Glycneg	322
661	MOMP_serE_ssHA2_C2S_Glycneg	323
662	MOMP_serE_ssHA2_C2S_Glycneg	324
663	MOMP_serF_FL_ssHA1	880
664	MOMP_serF_FL_ssHA1	326
665	MOMP_serF_FL_ssHA1_C2S	327
666	MOMP_serF_FL_ssHA1_C2S	328
667	MOMP_serF_FL_ssHA1_Glycneg	329

668	MOMP_serF_FL_ssHA1_Glycneg	330
669	MOMP_serF_FL_ssHA1_C2S_Glycneg	331
670	MOMP_serF_FL_ssHA1_C2S_Glycneg	332
671	MOMP_serF_FL_ssHA2	333
672	MOMP_serF_FL_ssHA2	334
673	MOMP_serF_FL_ssHA2_C2S	335
674	MOMP_serF_FL_ssHA2_C2S	336
675	MOMP_serF_FL_ssHA2_Glycneg	337
676	MOMP_serF_FL_ssHA2_Glycneg	338
677	MOMP_serF_FL_ssHA2_C2S_Glycneg	339
678	MOMP_serF_FL_ssHA2_C2S_Glycneg	340
679	MOMP_serG_FL_ssHA1	881
680	MOMP_serG_FL_ssHA1	342
681	MOMP_serG_FL_ssHA1_C2S	343
682	MOMP_serG_FL_ssHA1_C2S	874
683	MOMP_serG_FL_ssHA1_Glycneg	345
684	MOMP_serG_FL_ssHA1_Glycneg	346
685	MOMP_serG_FL_ssHA1_C2S	347
686	MOMP_serG_FL_ssHA1_C2S	348
687	MOMP_serG_FL_ssHA2	349
688	MOMP_serG_FL_ssHA2	350
689	MOMP_serG_FL_ssHA2_C2S	351
690	MOMP_serG_FL_ssHA2_C2S	352
691	MOMP_serG_FL_ssHA2_Glycneg	353
692	MOMP_serG_FL_ssHA2_Glycneg	354
693	MOMP_serG_FL_ssHA2_C2S	355
694	MOMP_serG_FL_ssHA2_C2S	356
695	Hirep2_patent_ssHA1 [CO1] PolyA	357
696	Hirep2_patent_ssHA1 [CO2] PolyA	358
697	Hirep2_patent_ssHA1_TMB1 [CO1] PolyA	359
698	Hirep2_patent_ssHA1_TMB1 [CO2] PolyA	360
699	Hirep2_patent_ssHA1_Glycneg [CO1] PolyA	361
700	Hirep2_patent_ssHA1_Glycneg [CO2] PolyA	362
701	Hirep2_patent_ssHA1_TMB1_Glycneg [CO1] PolyA	363
702	Hirep2_patent_ssHA1_TMB1_Glycneg [CO2] PolyA	364
703	CTH522_patent_ssHA1 [CO1] PolyA	365
704	CTH522_patent_ssHA1 [CO2] PolyA	366
705	CTH522_patent_ssHA1_Glycneg [CO1] PolyA	367
706	CTH522_patent_ssHA1_Glycneg [CO2] PolyA	368
707	CT443_ssHA1 PolyA	369
708	CT443_ssHA1 PolyA	370
709	CT443_ssHA1_Glycneg PolyA	371
710	CT443_ssHA1_Glycneg PolyA	372
711	CT584_ssHA1 PolyA	373
712	CT584_ssHA1 PolyA	374
713	CT584_ssHA1_C2S PolyA	375
714	CT584_ssHA1_C2S PolyA	376
715	CT584_ssHA1_Glycneg PolyA	377
716	CT584_ssHA1_Glycneg PolyA	378
717	CT584_ssHA1_Glycneg_C2S PolyA	379
718	CT584_ssHA1_Glycneg_C2S PolyA	380
719	CT600_trunc_ssHA1 PolyA	381
720	CT600_trunc_ssHA1 PolyA	382

721	CT600_trunc_ssHA1_Glycneg PolyA	383
722	CT600_trunc_ssHA1_Glycneg PolyA	384
723	CT812_pass-domain_ssHA1 PolyA	385
724	CT812_pass-domain_ssHA1 PolyA	386
725	CT812_pass-domain_ssHA1_C2S PolyA	387
726	CT812_pass-domain_ssHA1_C2S PolyA	388
727	CT812_ext-pass-domain_ssHA1 PolyA	389
728	CT812_ext-pass-domain_ssHA1 PolyA	390
729	CT812_ext-pass-domain_ssHA1_C2S PolyA	391
730	CT812_ext-pass-domain_ssHA1_C2S PolyA	392
731	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1 CO1	393
732	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1 CO2	394
733	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1_C2S CO1	395
734	CT812_pass-domain_ssHA1_HPX1-2_HPX2-1_C2S CO2	396
735	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3_C2S CO1	397
736	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3_C2S CO2	398
737	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1 CO1	399
738	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1 CO2	400
739	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3 CO1	401
740	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3 CO2	402
741	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1 CO1	403
742	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1 CO2	404
743	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3 CO1	405
744	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3 CO2	406
745	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3_C2S CO1	407
746	CT812_pass-domain_ssHA1_HPX1-2_HPX2-3_C2S CO2	408
747	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1_C2S CO1	409
748	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1_C2S CO2	410
749	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3 CO1	411
750	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3 CO2	412
751	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1_C2S CO1	413
752	CT812_pass-domain_ssHA1_HPX1-1_HPX2-1_C2S CO2	414
753	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3_C2S CO1	415
754	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-3_C2S CO2	416
755	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3_C2S CO1	417
756	CT812_pass-domain_ssHA1_HPX1-1_HPX2-3_C2S CO2	418
757	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3 CO1	419
758	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-3 CO2	420
759	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1 CO1	421
760	CT812_ext-pass-domain_ssHA1_HPX1-2_HPX2-1 CO2	422
761	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1_C2S CO1	423
762	CT812_ext-pass-domain_ssHA1_HPX1-1_HPX2-1_C2S CO2	424
763		425
764		426
765		427
766		428
767		429
768		430
769		431
770		432
771		433
772		434
773		435

774		436
775		437
776		438
777		439
778		440
779		441
780		442
790		452
791		453
792		454
793		455
794		456
795		457
796		458
797		861
800		536
801		537
802		538

CLAIMS

1. A composition comprising
 - (i) a nucleic acid comprising a nucleotide sequence encoding a modified Major Outer Membrane Protein (MOMP) polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia trachomatis* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences;
 - (ii) a nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia trachomatis* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia trachomatis* MOMP VD sequences of different serovars of *Chlamydia trachomatis*;
 - (iii) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia trachomatis* CT443 polypeptide; and
 - (iv) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia trachomatis* CT584 polypeptide.
2. A nucleic acid comprising a nucleotide sequence encoding a modified Major Outer Membrane Protein (MOMP) polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences, optionally wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.
3. A modified Major Outer Membrane Protein (MOMP) polypeptide having an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences, optionally wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.
4. The composition of claim 1, the nucleic acid of claim 2, or the modified MOMP polypeptide of claim 3, wherein the non-native loop sequence is no more than 40% identical to any native *Chlamydia sp.* MOMP VD (VD1, VD2, VD3, or VD4) sequence of any serovar (e.g., serovars D, E, F, or G of *C. trachomatis*).
5. The composition of claim 1 or 4, the nucleic acid of claim 2 or 4, or the modified MOMP polypeptide of claim 3 or 4, wherein the modified MOMP polypeptide:
 - (1) does not comprise a native *Chlamydia sp.* MOMP variable domain between the two or more conserved domain sequences; and/or

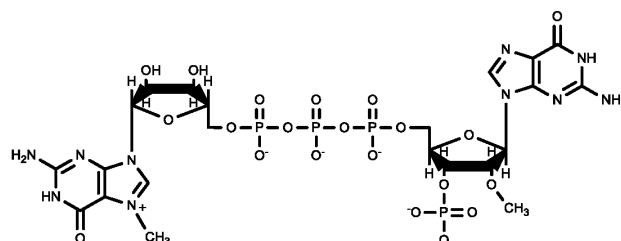
- (2) comprises five conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein (i) the modified MOMP polypeptide comprises all five full-length conserved domains of a native *Chlamydia sp.* MOMP polypeptide; and/or (ii) the conserved domains of the modified MOMP polypeptide collectively have at least 95% sequence identity to the conserved domains of a native MOMP polypeptide (e.g., serovar E MOMP).
6. The composition of any one of claims 1, 4 or 5, the nucleic acid of any one of claims 2, 4 or 5 or the modified MOMP polypeptide of any one of claims 3-5, wherein
- (1) the modified MOMP polypeptide comprises a non-native loop sequence between each of the conserved domain sequences, e.g., wherein the modified MOMP polypeptide comprises four non-native loop sequences and does not comprise any native *Chlamydia sp.* MOMP variable domains between the conserved domain sequences; and/or
- (2) the non-native loop sequence is between 3 and 30 amino acids in length, e.g., between 4 and 20 amino acids in length.
7. The composition of any one of claims 1 or 4-6, the nucleic acid of any one of claims 2 or 4-6, or the modified MOMP polypeptide of any one of claims 3-6, wherein the modified MOMP polypeptide comprises:
- (1) all five conserved domain sequences of a *Chlamydia sp.* MOMP polypeptide of *C. trachomatis* of any serovar, and
- (2) four non-native loop sequences, wherein a non-native loop sequence is located between each of the conserved domain sequences, and wherein the modified MOMP polypeptide does not comprise any native *Chlamydia sp.* MOMP variable domains between any of the conserved domain sequences,
- further wherein the non-native loop sequences are between 3 and 30 amino acids in length and no more than 40% identical to any native *Chlamydia sp.* MOMP VD (VD1, VD2, VD3, or VD4) sequences of any serovar.
8. The composition of any one of claims 1 or 4-7, the nucleic acid of any one of claims 2 or 4-7 or the modified MOMP polypeptide of any one of claims 3-7, wherein
- (i) a non-native loop sequence replacing VD1 comprises a sequence according to SEQ ID NO: 462 or 466 (e.g., SEQ ID NO:462);
- (ii) a non-native loop sequence replacing VD2 comprises a sequence according to SEQ ID NO: 463 or 467 (e.g., SEQ ID NO: 463);

(iii) a non-native loop sequence replacing VD3 comprises a sequence according to SEQ ID NOs 464 or 468 (e.g., SEQ ID NO: 464); and/or

(iv) a non-native loop sequence replacing VD4 comprises a sequence according to SEQ ID NO: 465 or 469 (e.g., SEQ ID NO: 465),

e.g., wherein the modified MOMP polypeptide comprises four non-native loop sequences according to SEQ ID NOs 462, 463, 464, and 465 in place of VD1, VD2, VD3, and VD4, respectively.

9. The composition of any one of claims 1 or 4-8, the nucleic acid of any one of claims 2 or 4-8 or the modified MOMP polypeptide of any one of claims 3-8, wherein the modified MOMP polypeptide comprises a sequence according to any one of SEQ ID NOs: 486-489 (e.g., SEQ ID NO: 486) or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto.
10. The composition of any one of claims 1 or 4-9 or the nucleic acid of any one of claims 2 or 4-9, wherein
 - (1) the modified MOMP polypeptide further comprises a secretion signal peptide sequence, optionally wherein the secretion signal peptide sequence comprises a secretion signal peptide sequence of HA protein of influenza A virus, e.g., wherein the secretion signal peptide sequence comprises a sequence according to SEQ ID NO: 187 or SEQ ID NO: 188; and/or
 - (2) the nucleic acid comprises a nucleotide sequence according to any one of SEQ ID NO: 551-566 (e.g., SEQ ID NO: 551) or a sequence that has at least 50% identity thereto.
11. The composition of any one of claims 1 or 4-10 or the nucleic acid of any one of claims 2 or 4-10, wherein the nucleic acid is a messenger RNA (mRNA), optionally wherein
 - (i) the mRNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (poly(A)) sequence;
 - (ii) the mRNA comprises at least one chemical modification, e.g., wherein the chemical modification comprises N1-methylpseudouridine, optionally wherein the chemical modification comprises N1-methylpseudouridine in place of every uridine; and/or
 - (iii) the mRNA is a self-replicating mRNA or a non-replicating mRNA, e.g., a non-replicating mRNA.
12. The composition or the nucleic acid of claim 11, wherein the mRNA comprises or consists of (e.g., consists of) the following structural elements:
 - a 5' cap, e.g., a cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 213;
- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- a polyA tail,

optionally wherein the mRNA is chemically modified and wherein the chemical modification comprises or consists of (e.g., consists of) N1-methylpseudouridine in place of every uridine.

13. A composition comprising

- (1) the nucleic acid of any one of claims 2 or 4-12; or.
- (2) the polypeptide of any one of claims 3-9,

preferably wherein the composition is an immunogenic composition.

14. The composition of claim 13, wherein the composition is as defined in (1) and the composition further comprises:

- (i) a nucleic acid (e.g., mRNA) that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*; and/or
- (ii) one or more (e.g., one, two, three or four) of:
 - (a) a nucleic acid (e.g., mRNA) that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide;
 - (b) a nucleic acid (e.g., mRNA) that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide;
 - (c) a nucleic acid (e.g., mRNA) that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide; and

- (d) a nucleic acid (e.g., mRNA) that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide,

optionally wherein the composition comprises:

the nucleic acid of (i) and the nucleic acid of (ii)(a);

the nucleic acid of (i) and the nucleic acid of (ii)(b); or

the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b);

e.g., wherein the composition comprises the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b).

the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b).

15. The composition of any one of claims 1 or 14, wherein

(A) one or more nucleic acids is a mRNA, optionally wherein

(1) the mRNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (poly(A)) sequence;

(2) the mRNA comprises at least one chemical modification, e.g., wherein the chemical modification comprises N1-methylpseudouridine, optionally wherein the chemical modification comprises N1-methylpseudouridine in place of every uridine; and/or

(3) the mRNA is a self-replicating mRNA or a non-replicating mRNA, e.g., a non-replicating mRNA; and/or

(B) the composition further comprises a lipid nanoparticle (LNP), optionally wherein the nucleic acid is encapsulated in the LNP.

16. The composition of claim 13, wherein the composition is as defined in (2) and the composition further comprises:

(i) a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*; and/or

(ii) one or more (e.g., one, two, three or four) of:

(a) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT443 polypeptide;

(b) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT584 polypeptide;

(c) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT600 polypeptide; or

(d) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT812 polypeptide,

optionally wherein the composition comprises

the polypeptide of (i) and the polypeptide of (ii)(a); or

the polypeptide of (i) and the polypeptide of (ii)(b); or

the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),

e.g., wherein the composition comprises the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b).

17. The composition of any one of claims 1, 4-12, or 14-16, wherein the chimeric MOMP VD polypeptide comprises conserved domain sequence portions of a native *Chlamydia sp.* MOMP polypeptide flanking each of the two or more MOMP VD sequences, optionally wherein the conserved domain sequence portions are portions of conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide flanking the VD in its native *Chlamydia sp.* MOMP polypeptide, further optionally wherein each conserved domain sequence portion comprises between 3 and 30 amino acid residues of a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, and wherein the between 3 and 30 amino acid residues are immediately adjacent to the VD sequence in its native *Chlamydia sp.* MOMP polypeptide.
18. The composition of any one of claims 1, 4-12, or 14-17, wherein the chimeric MOMP VD polypeptide comprises
- (1) MOMP VD sequences of four different serovars, e.g., wherein the different serovars are serovars D, E, F, or G of *C. trachomatis*;
 - (2)
 - (i) two MOMP VD1 sequences of different serovars of the *Chlamydia sp.* and/or
 - (ii) two MOMP VD2 sequences of different serovars of the *Chlamydia sp.*; and/or
 - (iii) one MOMP VD3 sequence; and/or
 - (iv) two MOMP VD4 sequences of different serovars of the *Chlamydia sp.*,

optionally wherein the different serovars are selected from serovars D, E, F or G of *C. trachomatis*; and/or

(3) one MOMP VD sequence of serovar D or E of *C. trachomatis* and one MOMP VD sequence of serovar F or G of *C. trachomatis*.

19. The composition of any one of claims 1, 4-12, or 14-18, wherein the chimeric MOMP VD polypeptide comprises at least one (e.g., four) of (1)-(4):

(1) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, or a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F or G (e.g., a MOMP VD1 sequence of serovar E and a MOMP VD1 sequence of serovar G); and/or

(2) a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, or a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G (e.g., a MOMP VD2 sequence of serovar D and a MOMP VD2 sequence of serovar G); and/or

(3) a MOMP VD3 sequence from serovar G or a VD3 sequence from serovar F (e.g., serovar F); and/or

(4) a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G, or a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F (e.g., a MOMP VD4 sequence of serovar D and a MOMP VD4 sequence of serovar F),

wherein the serovars D, E, F and G are of *C. trachomatis*.

optionally wherein the chimeric MOMP VD polypeptide comprises:

(i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, a MOMP VD3 sequence from serovar G, a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G; or

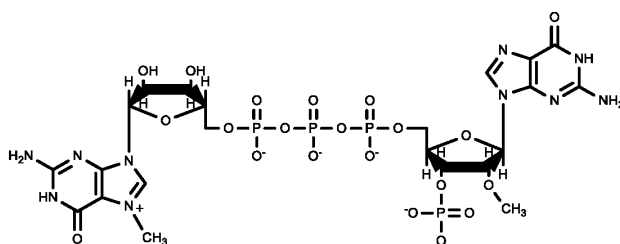
(ii) a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G, a MOMP VD3 sequence from serovar F, a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F,

e.g., wherein the chimeric MOMP VD polypeptide comprises MOMP VD sequences according to (ii).

20. The composition of any one of claims 1, 4-12 or 14-19, wherein

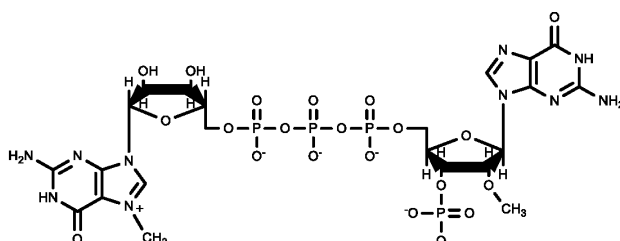
- (1) the chimeric MOMP VD polypeptide comprises a sequence according to any one of SEQ ID NO: 490-505 (e.g., SEQ ID NO: 503) or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
 - (2) the nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia* sp. MOMP VD polypeptide comprises a nucleotide sequence according to SEQ ID NO: 567-630 (e.g., SEQ ID NO: 617) or a sequence that has at least 50% identity thereto;
 - (3) the *Chlamydia* sp. CT443 polypeptide comprises a sequence according to SEQ ID NO: 507-508 (e.g., SEQ ID NO: 507) or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
 - (4) the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 707-710 (e.g., SEQ ID NO: 707) or a sequence that has at least 50% (e.g., at least 75%) identity thereto;
 - (5) the *Chlamydia* sp. CT584 polypeptide comprises a sequence according to any one of SEQ ID NO: 509-512 (e.g., SEQ ID NO: 510) or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
 - (6) the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 711-718 (e.g., SEQ ID NO: 715) or a sequence that has at least 50% (e.g., at least 75%) identity thereto;
 - (7) the *Chlamydia* sp. CT600 polypeptide comprises a sequence according to any one of SEQ ID NO: 513-514 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
 - (8) the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia* sp. CT600 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 719-722 or a sequence that has at least 50% (e.g., at least 75%) identity thereto;
 - (9) the *Chlamydia* sp. CT812 polypeptide comprises a sequence according to any one of SEQ ID NO: 515-535 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto; and/or
 - (10) the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia* sp. CT812 polypeptide comprises a nucleotide sequence according to any one of SEQ ID NO: 723-762 or a sequence that has at least 50% (e.g., at least 75%) identity thereto.
21. The composition of any one of claims 1, 4-12, 14, 15 or 17-20, wherein
- (1) the nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia* sp. CT443 polypeptide is a mRNA and the mRNA comprises or consists of (e.g., consists of) the following structural elements:

- a 5' cap, e.g., a 5' cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
 - a protein coding region having the nucleic acid sequence according to SEQ ID NO: 369;
 - a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
 - a polyA tail;
- (2) the nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide is a mRNA and the mRNA comprises or consists of (e.g., consists of) the following structural elements:

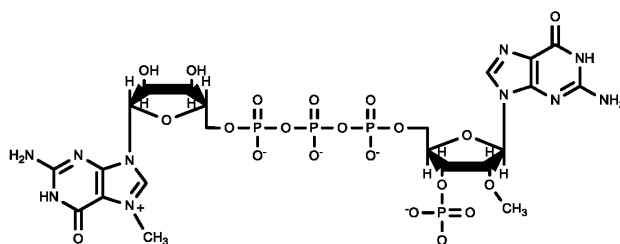
- a 5' cap, e.g., a 5' cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 377;
- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- a polyA tail; and/or

- (3) the nucleic acid comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide is a mRNA and the mRNA comprises or consists of (e.g., consists of) the following structural elements:

- a 5' cap, e.g., a 5' cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 870;
- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- a polyA tail.

22. The composition of any one of claims 1, 4-12, 14, 15 or 17-21, wherein the composition comprises:

- (1) the nucleic acid (e.g., mRNA) of claim 10(2), wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of claim 12);

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(3)); and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(1)); or
- (2) the nucleic acid (e.g., mRNA) of claim 10(2), wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of claim 12);

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia* sp. MOMP VD polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(3)); and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 715 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(2)); or

- (3) the nucleic acid (e.g., mRNA) of claim 10(2), wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of claim 12);

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia* sp. MOMP VD polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(3));

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(1)); and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 715 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(2)); or

- (4) the nucleic acid (e.g., mRNA) of claim 9, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia* sp. MOMP VD polypeptide, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the encoding the *Chlamydia* sp. CT443 polypeptide

comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

- (5) the nucleic acid (e.g., mRNA) of claim 9, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, wherein the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

- (6) the nucleic acid (e.g., mRNA) of claim 9, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the chimeric *Chlamydia sp.* MOMP VD polypeptide, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, wherein the encoding the *Chlamydia sp.* CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, wherein the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto,

e.g., wherein the composition comprises the nucleic acids as defined in (3) or the nucleic acids as defined in (6).

23. The composition of any one of claims 16-20, wherein the composition comprises:

- (1) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the *Chlamydia sp.* CT443 polypeptide that comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or
- (2) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the *Chlamydia sp.* CT584 polypeptide that comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or
- (3) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto;

the *Chlamydia sp.* CT443 polypeptide that comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the *Chlamydia sp.* CT584 polypeptide that comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto,

e.g., wherein the composition comprises the polypeptides as defined in (3).

24. A combination comprising a nucleic acid of any one of claims 2 or 4-12 and:

- (i) a nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*; and/or
- (ii) one or more (e.g., one, two, three or four) of:

- (a) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide;
- (b) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide;
- (c) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide; or
- (d) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide,

optionally wherein the combination comprises

the nucleic acid of (i) and the nucleic acid of (ii)(a);

the nucleic acid of (i) and the nucleic acid of (ii)(b); or

the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b),

e.g., wherein the combination comprises the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b),

optionally wherein the nucleic acids are as defined in claim 22,

further optionally wherein the nucleic acids are present in the same composition (e.g., in the same composition).

25. A combination comprising the polypeptide of any one of claims 3-9 and:

- (i) a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*; and/or
- (ii) one or more (e.g., one, two, three or four) of:
 - (a) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT443 polypeptide;
 - (b) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT584 polypeptide;
 - (c) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT600 polypeptide; or

(d) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT812 polypeptide,

optionally wherein the combination comprises

the polypeptide of (i) and the polypeptide of (ii)(a);

the polypeptide of (i) and the polypeptide of (ii)(b); or

the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),

e.g., wherein the combination comprises the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),

optionally wherein the polypeptides are as defined in claim 23,

further optionally wherein polypeptides are present in the same composition or in two or more separate compositions (e.g., in the same composition).

26. A nucleic acid that comprises a nucleotide sequence encoding a chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*, optionally wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.
27. A chimeric *Chlamydia sp.* MOMP variable domain (VD) polypeptide, wherein the chimeric MOMP VD polypeptide comprises an amino acid sequence comprising two or more *Chlamydia sp.* MOMP VD sequences of different serovars of the *Chlamydia sp.*, optionally wherein the *Chlamydia sp.* is *Chlamydia trachomatis*.
28. The nucleic acid of claim 26 or the chimeric MOMP VD polypeptide of claim 27, wherein
 - (a) the chimeric MOMP VD polypeptide comprises conserved domain sequence portions of a native *Chlamydia sp.* MOMP polypeptide flanking each of the two or more MOMP VD sequences;
 - (b) the chimeric MOMP VD polypeptide comprises MOMP VD sequences of four different serovars, optionally wherein the different serovars are selected from serovars D, E, F or G of *C. trachomatis*.
29. The nucleic acid of claim 26 or 28 or the chimeric MOMP VD polypeptide of claim 27 or 28, wherein the chimeric MOMP VD polypeptide comprises:
 - (1)
 - (i) two MOMP VD1 sequences of different serovars of the *Chlamydia sp.* and/or
 - (ii) two MOMP VD2 sequences of different serovars of the *Chlamydia sp.*; and/or

(iii) one MOMP VD3 sequence; and/or

(iv) two MOMP VD4 sequences of different serovars of the *Chlamydia sp.*,

optionally wherein the different serovars are selected from serovars D, E, F, or G of *C. trachomatis*; and/or

(2) one MOMP VD sequence of serovar D or E of *C. trachomatis* and one MOMP VD sequence of serovar F or G of *C. trachomatis*,

30. The nucleic acid of claim 29 or the chimeric MOMP VD polypeptide of claim 29, wherein the chimeric MOMP VD polypeptide comprises at least one (e.g., four) of (i)-(iv):

(i) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, or a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F or G (e.g., a MOMP VD1 sequence of serovar E and a MOMP VD1 sequence of serovar G); and/or

(ii) a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, or a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G (e.g., a MOMP VD2 sequence of serovar D and a MOMP VD2 sequence of serovar G); and/or

(iii) a MOMP VD3 sequence from serovar G or a VD3 sequence from serovar F (e.g., serovar F); and/or

(iv) a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G, or a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F (e.g., a MOMP VD4 sequence of serovar D and a MOMP VD4 sequence of serovar F),

wherein the serovars D, E, F or G are of *C. trachomatis*,

optionally wherein the chimeric MOMP VD polypeptide comprises:

(1) a MOMP VD1 sequence from serovar D and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar E and a MOMP VD2 sequence from serovar F, a MOMP VD3 sequence from serovar G, a MOMP VD4 sequence from serovar E and a MOMP VD4 sequence from serovar G; or

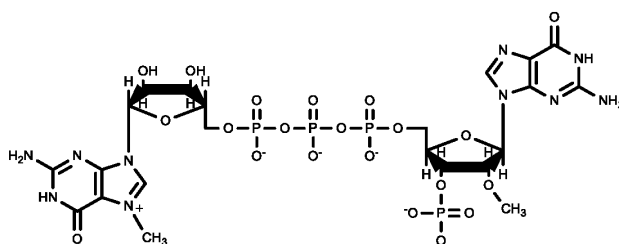
(2) a MOMP VD1 sequence from serovar E and a MOMP VD1 sequence from serovar F, a MOMP VD2 sequence from serovar D and a MOMP VD2 sequence from serovar G, a MOMP VD3 sequence from serovar F, a MOMP VD4 sequence from serovar D and a MOMP VD4 sequence from serovar F,

wherein the serovars D, E, F or G are of *C. trachomatis*.

e.g., wherein the chimeric MOMP VD polypeptide comprises MOMP VD sequences according to (2).

31. The nucleic acid of any one of claim 28-30 or the chimeric MOMP VD polypeptide of any one of claims 28-30, wherein
 - (a) the conserved domain sequence portions are portions of conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide flanking the VD in its native *Chlamydia sp.* MOMP polypeptide; and/or
 - (b) each conserved domain sequence portion comprises between 3 and 30 amino acid residues of a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, wherein the between 3 and 30 amino acid residues are immediately adjacent to the VD sequence in its native *Chlamydia sp.* MOMP polypeptide.
32. The nucleic acid of any one of claims 26 or 28-31 or the chimeric MOMP VD polypeptide of any one of claims 27-31, wherein the chimeric MOMP VD polypeptide comprises a sequence according to any one of SEQ ID NO: 490-505 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto, e.g., wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto.
33. The nucleic acid of any one of claims 26 or 28-32, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 567-630 or a sequence that has at least 50% (e.g., at least 75%) identity thereto, e.g., wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto.
34. The nucleic acid of any one of claims 26 or 28-33, wherein the nucleic acid is a messenger RNA (mRNA), optionally wherein
 - (i) the mRNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (poly(A)) sequence;
 - (ii) the mRNA comprises at least one chemical modification, e.g., wherein the chemical modification comprises N1-methylpseudouridine, optionally wherein the chemical modification comprises N1-methylpseudouridine in place of every uridine; and/or
 - (iii) the mRNA is a self-replicating mRNA or a non-replicating mRNA, e.g., a non-replicating mRNA.
35. The nucleic acid of any one of claims 26 or 28-34, wherein the mRNA comprises or consists of (e.g., consists of) the following structural elements:

- a 5' cap, e.g., a 5' cap with the following structure:



- a 5' untranslated region (5' UTR) having the nucleic acid sequence according to SEQ ID NO: 838;
- a protein coding region having the nucleic acid sequence according to SEQ ID NO: 870;
- a 3' untranslated region (3' UTR) having the nucleic acid sequence according to SEQ ID NO: 839; and
- a polyA tail,

optionally wherein the mRNA is chemically modified and wherein the chemical modification comprises or consists of (e.g., consists of) N1-methylpseudouridine in place of every uridine.

36. A composition comprising the nucleic acid of any one of claims 26 or 28-35, preferably wherein the composition is an immunogenic composition.
37. A composition comprising the chimeric MOMP VD polypeptide of any one of claims 27-32, preferably wherein the composition is an immunogenic composition, e.g., wherein the composition comprises an adjuvant.
38. The composition of claim 36, wherein the composition further comprises:
 - (i) a nucleic acid that comprises a nucleotide sequence encoding a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia* sp. MOMP polypeptide and a non-native loop sequence between the conserved domain sequences; and/or
 - (ii) one or more (e.g., one, two, three or four) of:
 - (a) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia* sp. CT443 polypeptide;
 - (b) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia* sp. CT584 polypeptide;

- (c) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide; or
 - (d) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide,
optionally wherein the composition comprises
the nucleic acid of (i) and the nucleic acid of (ii)(a);
the nucleic acid of (i) and the nucleic acid of (ii)(b); or
the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b),
e.g., wherein the composition comprises the nucleic acid of (i), the nucleic acid of (ii)(a)
and the nucleic acid of (ii)(b).
39. The composition of claim 38, wherein one or more nucleic acids is a mRNA, optionally wherein
- (i) the mRNA comprises at least one 5' untranslated region (5' UTR), at least one 3' untranslated region (3' UTR), and/or at least one polyadenylation (poly(A)) sequence;
 - (ii) the mRNA comprises at least one chemical modification, e.g., wherein the chemical modification comprises N1-methylpseudouridine, optionally wherein the chemical modification comprises N1-methylpseudouridine in place of every uridine; and/or
 - (iii) the mRNA is a self-replicating mRNA or a non-replicating mRNA, e.g., a non-replicating mRNA.
40. The composition of claim 38 or 39, wherein
- (1) the nucleic acid that comprises a nucleotide sequence encoding the modified MOMP polypeptide is as defined in any one of claims 2 or 4-12; and/or
 - (2) any one of the nucleic acid that comprises a nucleotide sequence encoding the *Chlamydia sp.* CT443 polypeptide, the nucleic acid that comprises a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, the nucleic acid that comprises a nucleotide sequence encoding the *Chlamydia sp.* CT600 polypeptide or the nucleic acid that comprises a nucleotide sequence encoding the *Chlamydia sp.* CT812 polypeptide is as defined in any one of claims 20-22.
41. The composition of any one of claims 38-40, wherein the composition comprises:

- (1) the nucleic acid (e.g., mRNA) of claim 10(2), wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of claim 12);

the nucleic acid (e.g., mRNA) of claim 33, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 35); and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(1)); or
- (2) the nucleic acid (e.g., mRNA) of claim 10(2), wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of claim 12);

the nucleic acid (e.g., mRNA) of claim 33, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 35); and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 715 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(2)); or
- (3) the nucleic acid (e.g., mRNA) of claim 10(2), wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 551 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is a mRNA of claim 12);

the nucleic acid (e.g., mRNA) of claim 33, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 617 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 35);

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the nucleic acid comprises a nucleotide sequence according to SEQ ID NO: 707 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(1)); and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide, wherein the nucleic acid comprises a nucleotide sequence

according to SEQ ID NO: 715 or a sequence that has at least 50% (e.g., at least 75%) identity thereto (e.g., wherein the nucleic acid is the mRNA of claim 21(2)); or

- (4) the nucleic acid (e.g., mRNA) of claim 9, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) of claim 32, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the *Chlamydia* sp. CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

- (5) the nucleic acid (e.g., mRNA) of claim 9, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) of claim 32, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia* sp. CT584 polypeptide, wherein the *Chlamydia* sp. CT584 polypeptide comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or

- (6) the nucleic acid (e.g., mRNA) of claim 9, wherein the modified MOMP polypeptide comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) of claim 32, wherein the chimeric MOMP VD polypeptide comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto;

the nucleic acid (e.g., mRNA) comprising a nucleotide sequence encoding the *Chlamydia* sp. CT443 polypeptide, wherein the *Chlamydia* sp. CT443 polypeptide comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and

the nucleic acid comprising a nucleotide sequence encoding the *Chlamydia sp.* CT584 polypeptide, wherein the *Chlamydia sp.* CT584 polypeptide comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto,

e.g., wherein the composition comprises the nucleic acids as defined in (3) or the nucleic acids as defined in (6).

42. The composition of claim 37, wherein the composition further comprises:
- (i) a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences; and/or
 - (ii) one or more (e.g., one, two, three, or four) of:
 - (a) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT443 polypeptide;
 - (b) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT584 polypeptide;
 - (c) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT600 polypeptide; or
 - (d) a polypeptide comprising an amino acid sequence of a *Chlamydia sp.* CT812 polypeptide,optionally wherein the composition comprises:
 - the polypeptide of (i) and the polypeptide of (ii)(a); or
 - the polypeptide of (i) and the polypeptide of (ii)(b); or
 - the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),e.g., wherein the composition comprises the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b).

43. The composition of claim 42, wherein

- (1) the modified MOMP polypeptide is as defined in any one of claims 3-9; and/or

- (2) any one of the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide or the *Chlamydia sp.* CT812 polypeptide is as defined in claim 20 or 22.
44. The composition of claim 42 or 43, wherein the composition comprises:
- (1) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and
- the *Chlamydia sp.* CT443 polypeptide that comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or
- (2) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and
- the *Chlamydia sp.* CT584 polypeptide that comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; or
- (3) the modified MOMP polypeptide that comprises a sequence according to SEQ ID NO: 486 or a sequence that has at least 70% (e.g., at least 90 or 95%) identity thereto;
- the chimeric *Chlamydia sp.* MOMP VD polypeptide that comprises a sequence according to SEQ ID NO: 503 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto;
- the *Chlamydia sp.* CT443 polypeptide that comprises a sequence according to SEQ ID NO: 507 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto; and
- the *Chlamydia sp.* CT584 polypeptide that comprises a sequence according to SEQ ID NO: 510 or a sequence that has at least 75% (e.g., at least 90 or 95%) identity thereto,
- e.g., wherein the composition comprises the polypeptides as defined in (3).
45. A combination comprising a nucleic acid of any one of claims 26 or 28-35 and:
- (i) a nucleic acid that comprises a nucleotide sequence encoding a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP

polypeptide and a non-native loop sequence between the conserved domain sequences;
and/or

- (ii) one or more (e.g., one, two, three, or four) of:
 - (a) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT443 polypeptide;
 - (b) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT584 polypeptide;
 - (c) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT600 polypeptide; or
 - (d) a nucleic acid that comprises a nucleotide sequence encoding a *Chlamydia sp.* CT812 polypeptide,

optionally wherein the combination comprises

the nucleic acid of (i) and the nucleic acid of (ii)(a);

the nucleic acid of (i) and the nucleic acid of (ii)(b); or

the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b),

e.g., wherein the combination comprises the nucleic acid of (i), the nucleic acid of (ii)(a) and the nucleic acid of (ii)(b),

optionally wherein the nucleic acids are as defined in claim 41,

further optionally wherein the nucleic acids are present in the same composition (e.g., in the same composition).

46. A combination comprising a polypeptide of any one of claims 27-32 and:

- (i) a modified MOMP polypeptide, wherein the modified MOMP polypeptide has an amino acid sequence comprising two or more conserved domain sequences of a native *Chlamydia sp.* MOMP polypeptide and a non-native loop sequence between the conserved domain sequences; and/or
- (ii) one or more (e.g., one, two, three, or four) of:
 - (a) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT443 polypeptide;

(b) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT584 polypeptide;

(c) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT600 polypeptide; or

(d) a polypeptide comprising amino acid sequence of a *Chlamydia sp.* CT812 polypeptide,

optionally wherein the combination comprises

the polypeptide of (i) and the polypeptide of (ii)(a);

the polypeptide of (i) and the polypeptide of (ii)(b); or

the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),

e.g., wherein the combination comprises the polypeptide of (i), the polypeptide of (ii)(a) and the polypeptide of (ii)(b),

optionally wherein the polypeptides are as defined in claim 44,

further optionally wherein the polypeptides are present in the same composition or in two or more separate compositions.

47. The nucleic acid of any one of claims 2, 4-12, 26 or 28-35, the composition of any one of claims 1, 4-12, 13-15, 17-23, 36-41, or the combination of any one of claims 24 or 45, wherein:

- (i) the modified MOMP polypeptide further comprises a secretion signal peptide sequence;
- (ii) the chimeric MOMP VD polypeptide comprises a secretion signal peptide sequence; and/or
- (iii) the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide and the *Chlamydia sp.* CT812 polypeptide comprise a secretion signal peptide sequence,

optionally wherein the secretion signal peptide sequence is a viral secretion signal peptide sequence, optionally selected from the group consisting of: an influenza hemagglutinin (HA) secretion signal peptide sequence, a SARS CoV-2 spike secretion signal peptide sequence, a VZV gB secretion signal peptide sequence, a VZV gE secretion signal peptide sequence, a VZV gI secretion signal peptide sequence, a VZV gK secretion signal peptide sequence, a measles F-protein secretion signal peptide sequence, a rubella E1 protein secretion signal peptide sequence, a rubella E2 protein secretion signal peptide sequence, a mumps F-protein secretion signal peptide sequence, an Ebola GP protein secretion signal peptide sequence, and a smallpox 6kDa IC protein secretion

signal peptide sequence, optionally wherein the secretion signal peptide sequence comprises an amino acid sequence according to one of the SEQ ID NOs in Table 2 or Table 2.1,

e.g., wherein the secretion signal peptide sequence comprises a secretion signal peptide sequence of HA protein of influenza A virus, e.g., wherein the secretion signal peptide sequence comprises a sequence according to SEQ ID NO: 187 or SEQ ID NO: 188.

48. The nucleic acid of any one of claims 2, 4-12, 26, 28-35 or 47, the modified MOMP polypeptide of any one of claims 3-9, the chimeric MOMP VD polypeptide of any one of claims 27-32, the composition of any one of claims 1, 4-23, 36-44 or 47, or the combination of any one of claims 24, 25 or 45-47, wherein:
- (i) the modified MOMP polypeptide comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the single amino acid substitution is a substitution of cysteine with serine;
 - (ii) the chimeric MOMP VD polypeptide comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in a conserved domain sequence of a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the single amino acid substitution is a substitution of cysteine with serine; and/or
 - (iii) one or more of the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide or the *Chlamydia sp.* CT812 polypeptide comprises a single amino acid substitution at one or more (e.g., all) positions corresponding to a cysteine residue in the respective native *Chlamydia sp.* polypeptide, optionally wherein the single amino acid substitution is a substitution of cysteine with serine.
49. The nucleic acid of any one of claims 2, 4-12, 26, 28-35, 47 or 48, the modified MOMP polypeptide of any one of claims 3-9 or 48, the chimeric MOMP VD polypeptide of any one of claims 27-32 or 48, the composition of any one of claims 1, 4-23, 36-44, 47 or 48, or the combination of any one of claims 24, 25 or 45-48, wherein
- (i) the modified MOMP polypeptide comprises a mutation at one or more (e.g., all) positions corresponding to a glycosylation site, optionally an N-glycosylation site, in a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the mutation is a single amino acid substitution;
 - (ii) the chimeric MOMP VD polypeptide comprises a mutation at one or more (e.g., all) positions corresponding to a glycosylation site, optionally an N-glycosylation site, in a native *Chlamydia sp.* MOMP polypeptide, optionally wherein the mutation is a single amino acid substitution, e.g. wherein the chimeric MOMP VD polypeptide comprises a single amino acid

substitution at each of the amino acid residues corresponding to position 9 of SEQ ID NO: 9 (e.g., N to A substitution), position 11 of SEQ ID NO: 17 (e.g., T to A substitution), position 17 of SEQ ID NO: 6 (e.g., S to A substitution), positions 4 and 21 of SEQ ID NO: 18 (e.g., N to A and S to A substitutions, respectively), position 14 of SEQ ID NO: 8 (e.g., a T to A substitution) and position 14 in SEQ ID NO: 16 (e.g., T to A substitution); and/or

(iii) one or more of the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide or the *Chlamydia sp.* CT812 polypeptide comprises a mutation at one or more (e.g., all) positions corresponding to an N-glycosylation site in the respective native *Chlamydia sp.* polypeptide, optionally wherein the mutation is a single amino acid substitution, e.g., wherein the *Chlamydia sp.* CT584 polypeptide comprises a single amino acid substitution at a position corresponding to residue 11 of SEQ ID NO: 509 (e.g., a N to Q substitution).

50. The composition of any one of claims 1, 4-23, 36-44 or 47-49, or the combination of any one of claims 24, 25 or 45-49, wherein one or more of the *Chlamydia sp.* CT443 polypeptide, the *Chlamydia sp.* CT584 polypeptide, the *Chlamydia sp.* CT600 polypeptide or the *Chlamydia sp.* CT812 polypeptide comprises a heterologous transmembrane domain, optionally wherein the transmembrane domain sequence is selected from the group consisting of: an influenza hemagglutinin (HA) transmembrane domain sequence, a SARS CoV-2 spike transmembrane domain sequence, a VZV gB transmembrane domain sequence, a VZV gE transmembrane domain sequence, a VZV gI transmembrane domain sequence, a VZV gK transmembrane domain sequence, a measles F-protein transmembrane domain sequence, a rubella E1 protein transmembrane domain sequence, a rubella E2 protein transmembrane domain sequence, a mumps F-protein transmembrane domain sequence and an Ebola GP protein transmembrane domain sequence, optionally wherein the transmembrane domain comprises an amino acid sequence according to one of the SEQ ID NOs in Table 3, e.g., wherein the transmembrane domain comprises the sequence of a transmembrane domain of HA protein of influenza A virus, optionally wherein the transmembrane domain comprises a sequence according to SEQ ID NO: 813 or SEQ ID NO: 814.
51. The composition of any one of claims 1, 4-15, 17-23, 36-41, wherein the composition further comprises a lipid nanoparticle (LNP), optionally wherein the nucleic acid is encapsulated in the LNP.
52. The composition of claim 52, wherein the LNP comprises at least one cationic lipid, optionally wherein:

- (i) the cationic lipid is selected from the group consisting of OF-02, cKK-E10, OF-Deg-Lin, GL-HEPES-E3-E10-DS-3-E18-1, GL-HEPES-E3-E12-DS-4-E10, GL-HEPES-E3-E12-DS-3-E14, SM-102, ALC-0315, ATX-126, and IM-001 (e.g., GL-HEPES-E3-E12-DS-4-E10 or IM-001); and/or
 - (ii) the LNP further comprises a polyethylene glycol (PEG) conjugated (PEGylated) lipid, a cholesterol-based lipid, and a helper lipid, optionally wherein
 - the (PEGylated) lipid is DMG-PEG2000 or ALC-0159 (e.g., DMG-PEG2000);
 - the cholesterol-based lipid is cholesterol; and/or
 - the helper lipid is DOPE or DSPC.
53. The composition of claim 51 or 52, wherein the LNP comprises:
- (i) OF-02 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
 - (ii) cKK-E10 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
 - (iii) GL-HEPES-E3-E10-DS-3-E18-1 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
 - (iv) GL-HEPES-E3-E12-DS-4-E10 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
 - (v) GL-HEPES-E3-E12-DS-3-E14 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
 - (vi) DLin-MC3-DMA (MC3) at a molar ratio of 50%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 38.5%, and DSPC at a molar ratio of 10%;
 - (vii) IM-001 at a molar ratio of 40%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 28.5%, and DOPE at a molar ratio of 30%;
 - (viii) SM-102 at a molar ratio of 50%, DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 38.5%, and DSPC at a molar ratio of 10%;
 - (ix) ALC-0315 at a molar ratio of 46.3%, ALC-0159 at a molar ratio of 1.6%, cholesterol at a molar ratio of 42.7%, and DSPC at a molar ratio of 9.4%;
 - (x) ALC-0315 at a molar ratio of 47.4%, ALC-0159 at a molar ratio of 1.7%, cholesterol at a molar ratio of 40.9%, and DSPC at a molar ratio of 10%; or

- (xi) ATX-126 at a molar ratio of 50%; DMG-PEG2000 at a molar ratio of 1.5%, cholesterol at a molar ratio of 38.5%, and DSPC at a molar ratio of 10%,

e.g., wherein the LNP is as defined in (i), (ii), (iv) or (vii), e.g., wherein the LNP is as defined in (iv) or (vii).

54. The nucleic acid of any one of claims 2, 4-12, 26, 28-35 or 47-49, the modified MOMP polypeptide of any one of claims 3-9, 48 or 49, the chimeric MOMP VD polypeptide of any one of claims 27-32, 48 or 49, the composition of any one of claims 1, 4-23, 36-44, or 47-53, or the combination of any one of claims 24, 25 or 45-50, for use as a medicament.
55. The nucleic acid of any one of claims 2, 4-12, 26, 28-35 or 47-49, the modified MOMP polypeptide of any one of claims 3-9, 48 or 49, the chimeric MOMP VD polypeptide of any one of claims 27-32, 48 or 49, the composition of any one of claims 1, 4-23, 36-44, or 47-53, or the combination of any one of claims 24, 25 or 45-50 for use in treating or preventing a *Chlamydia sp.* infection, preferably wherein the infection is a *C. trachomatis* infection, e.g., a *C. trachomatis* genital infection.
56. A vaccine comprising the nucleic acid of any one of claims 2, 4-12, 26, 28-35 or 47-49, the modified MOMP polypeptide of any one of claims 3-9, 48 or 49, the chimeric MOMP VD polypeptide of any one of claims 27-32, 48 or 49, the composition of any one of claims 1, 4-23, 36-44, or 47-53, or the combination of any one of claims 24, 25 or 45-50.