The invention relates to a composition comprising a viral vector, the viral vector comprising nucleic acid having a polynucleotide sequence encoding the spike protein from the middle eastern respiratory syndrome coronavirus (MERS-CoV), characterised in that said viral vector is an adenovirus based vector. Suitably said adenovirus based vector is ChAdOx 1, and said spike protein is full length spike protein. More suitably said spike protein is present as a fusion with the tissue plasminogen activator (tPA) sequence in the order N-terminus - tPA - spike protein - C-terminus. The invention also relates to uses and methods.
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TI, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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Compositions and Methods for inducing an immune response

FIELD OF THE INVENTION

The invention relates to induction of immune responses, suitably protective immune responses, against Middle East respiratory syndrome coronavirus (MERS-CoV).

BACKGROUND TO THE INVENTION

The Middle East respiratory syndrome coronavirus (MERS-CoV) has infected more than 1900 humans since 2012. The syndrome ranges from asymptomatic and mild cases to severe pneumonia and death. The virus is believed to be circulating in dromedary camels without notable symptoms since the 1980s. Therefore, dromedary camels are considered the only animal source of infection. Neither antiviral drugs nor vaccines are approved for veterinary or medical use despite active research on this area, which is a problem in the art.

Middle East respiratory syndrome (MERS) is caused by a novel betacoronavirus (MERS-CoV) that was isolated in late 2012 in Saudi Arabia (1). The syndrome (MERS) is described as a viral infection that causes fever, cough, and/or shortness of breath and to a lesser extent gastrointestinal symptoms such as diarrhea (2). Severe disease from MERS-CoV infection can cause respiratory failure and organ failure, and cases can be fatal, especially in patients with co-morbidities such as diabetes and cardiac complications. However, the infection can be asymptomatic or mild in many cases (3-7). MERS-CoV has spread to 27 countries and infected more than 1900 humans with a mortality rate of 40% (2). Dromedary camels, especially juveniles, contract the infection and shed the virus, without notable symptoms of disease; this is now known to have been occurring since the early 1980s (8-13). The mechanism of camel to human transmission is still not clear, but several primary cases have been associated with camel contact, which is considered an important risk factor (14-16). Therefore, camels are being considered an intermediate host and one of the sources of MERS-CoV infection (8-13). Other livestock animals such as sheep, goats, cows, chicken, and horses have proved seronegative in many studies (17-20). Further, these animals did not productively contract MERS-CoV when they were inoculated experimentally (21, 22). Therefore, to date, dromedary camels are the only confirmed animal reservoir. There is currently no approved vaccine against MERS-CoV for camels or humans despite active vaccine research and development. A number of vaccine candidates have
been developed using various platforms and regimens and have been tested in several animal models (23). These all require multiple doses (administrations) to achieve any effect. Moreover, these can also induce immunopathology such as hypersensitivity.

No licensed vaccines or treatments are currently available for MERS-CoV infections. Ongoing disease control strategies have so far relied on minimising contact with dromedary camels, observing standard infection control measures to limit nosocomial transmission, contact tracing and quarantine. Addressing this unmet need for MERS-CoV interventions has been prioritised by the WHO for urgent action 4. A major gap remains in the understanding of key immune mechanisms responsible for protection from disease; whilst MERS-CoV infections elicit high titre neutralising antibody, these do not appear sufficient to provide long-term protection against re-infection 11-13.

Vaccination approaches using full-length S protein of MERS-CoV have proven problematic. Hotez et al. 2014 (Microbes and Infection 2016, pages 529 - 531) discusses calls for rapid development of a safe and effective MERS vaccine. It is disclosed at page 530 that use of subunit vaccines comprising the full spike protein of the severe acute respiratory syndrome (SARS) coronavirus caused eosinophilic immune enhancement in the lungs - a form of hypersensitivity that is very undesirable. The authors note efforts to develop a more restricted receptor binding domain (RBD) of the coronavirus spike protein as a recombinant vaccine against SARS. Such an approach is said to elicit highly effective cross-neutralising antibody responses against SARS in vaccinated animals, but the authors note a general unwillingness to follow this route for generating a MERS vaccine on account of the time and cost involved in this development and the accepted existence of uncertain risk factors (see page 530, left column, first full paragraph).

The unwillingness around development of a MERS vaccine as reported in Hotez et al is amply supported by the reported studies in related coronaviruses such as that responsible for severe acute respiratory syndrome (SARS-CoV). Jaume et al. 2012 (Hong Kong Med J, Volume 18, page 31) disclose enhancement of SARS-CoV infection by subunit vaccines comprising a recombinant native full length spike protein trimer (triSpike). This study raises very serious safety concerns over the use of full-length coronavirus spike proteins as antigens in vaccine compositions. Such concerns are supported by the findings reported by many research groups including Tseng et al. 2012 (PLoS ONE, Volume 7, document 635421) who discloses that immunisation with a SARS coronavirus vaccine leads to pulmonary immunopathology on challenge with the
SARS virus and advises caution in proceeding to application of a coronavirus vaccine in humans.

Efforts to avoid hypersensitivity reactions when immunising with proteins from other coronavirus pathogens have relied on new antigen-adjuvant combinations. Honda-Okubo et al. 2015 (Journal of Virology, Volume 89, page 2995) disclose SARS vaccines comprising inactivated virus or recombinant viral protein formulated with delta inulin adjuvants to ameliorate lung eosinophilic immunopathology. Iwata-Yoshikawa et al. 2014 (Journal of Virology, Volume 88, page 8597) disclose SARS vaccines comprising a UV-inactivated SARS virus together with Toll-like receptor (TLR) agonists including lipopolysaccharide, poly(U), and poly(I-C) (UV-V+TLR) to reduce excess eosinophilic infiltration in the lungs.

Other investigators have attempted to develop vaccines against coronaviruses using viral vectors. Weingartl et al. 2004 (Journal of Virology, Volume 78, page 12672) disclose that immunisation with modified vaccinia virus Ankara expressing the SARS-CoV spike protein is associated with enhanced hepatitis in ferrets.

Haagmans et al. 2016 (Science, Volume 351, page 77) discloses an orthopoxvirus based vaccine which reduces MERS-CoV viral RNA excretion after MERS-CoV infection in dromedary camels. The authors show that a modified vaccinia virus Ankara (MVA) vaccine expressing the MERS-CoV spike protein confers mucosal immunity in dromedary camels when it is administered in two doses following a prime-boost regimen, and no protective immunity is demonstrated.

Volz et al. 2015 (Journal of Virology, Volume 89, page 8651) replicates and extends the findings of Haagmans et al. in mice. This reference discloses "protective efficacy" of a recombinant MVA vaccine expressing the MERS-CoV spike protein delivered in two doses as a prime-boost regimen. Notably, the first or prime dose produced weak immune responses that only became protective after the second or boost vaccination ("prime-boost" vaccination i.m. in Figure 2).

Malczyk et al. 2015 (Journal of Virology, Volume 89, page 11654) disclose a MERS-CoV vaccine based on a recombinant measles virus vaccine platform that induced a protective immune response only after administration of two doses. No evidence of a protective effect after only a single dose is provided.
Thus, there exists no vaccine in the prior art capable of delivering protection against the MERS coronavirus after only a single dose of vaccine.

The present seeks to overcome problem(s) associated with the prior art.

SUMMARY OF THE INVENTION

We describe a combination which comprises a simian adenoviral vector (such as ChAdOxi) delivering a MERS-CoV antigen (the spike protein). This combination has surprising technical benefits including the absence of hypersensitivity type reactions after immunisation. A key benefit delivered by this new combination is the induction of protective immune response after only a single vaccine administration.

In addition, the inventors describe the optional incorporation of a leader sequence/secretory sequence such as the tissue plasminogen activator (tPA) amino acid sequence fused to the N-terminus of the MERS-CoV spike protein antigen. This triple combination (ChAdOxi + tPA + MERS spike protein) delivers enhanced immunogenicity. The inventors provide data demonstrating that a single dose of this combined construct delivers 5-log increase in the relevant immune responses - data demonstrating these advantages are provided in the Examples section below.

Thus, in one aspect the invention relates to a composition comprising a viral vector, the viral vector comprising nucleic acid having a polynucleotide sequence encoding the spike protein from the middle eastern respiratory syndrome coronavirus (MERS-CoV), characterised in that said viral vector is an adenovirus based vector.

Suitably said adenovirus based vector is ChAdOx 1.

Suitably said spike protein is full length spike protein.

Suitably said spike protein is present as a fusion with the tissue plasminogen activator (tPA) sequence. Suitably the fusion is in the order N-terminus - tPA - spike protein - C-terminus.

Suitably said spike protein has the amino acid sequence given in the MERS genome of GenBank accession number KJ650098.1.
Suitably said spike protein has the amino acid sequence given in GenBank accession number AHX71946.1.

Suitably the Middle East respiratory syndrome coronavirus may be isolate Camel/Qatar_2_20i4.

Suitably said spike protein has the amino acid sequence of SEQ ID NO: 1.

SEQ ID NO: 1 - GenBank accession number AHX71946.1

MIHSVFLMLFLLTPTESYVDVGPDSDKSVKSACIEVDIQQTFFDKTW
PRPIDVSKADGI YPQGQRTYSNITYQGLFPYQGDGMYVYSAHATGTPQKLFV
ANYSQVDKQFANGFVVRIGAANSTGTVI ISPSTSATIRKI YPAFLMGSSVGNFSDGK
MGFRFHNHTLLVLPPDGCTTLLARAFYCILEPRSGHCPAGNSHTFSATYHTPATDCSDGN
YNRNASLNSFKEYFNLRNCMTFMYTNITEDEILEWFGITQTAQGVHLFSRYVLYGG

NMFQFATLPVYDITKYYSI IPHS IRSIQSDRKAFAVYVYKLQPLTFLLDFSVDGYIR
RAICDGFLNLQHCSYESFDVODEYGVSVEFSEAKPSSQENAGVHVECDFSFPLLSGT
PPQVNFKRLVFTNCNYNLTKLLSFSVNDFTCQSISPAAIASNCYSSLILDFSYPL
SMKSDLSSAGISPISQFYQKQFSNSPTCLILATVPHNLTTIKPLKSYINKCSRLLS
DDRTEVPQVLVANQYSPCVSIVPSTVWEDGDYRKQLSLEGPGGWVLASGTVAMTEQ

LQMFGITVQYGTDDNSVCPKLEFANDTAKASQLGNCVEYSLGYGSGRQVFNCTAVG
VRQQRFVYDAYQNLVGYSSDGNYCLRCVSPVSVIYDGETKTTHATLFVSACEHI
SSTMSQYSRSTRSMKLRRDSTYPLQTPVGCVLQVNLSSLFVEDCKPLQLGSCLALPD
TPSTLTPRSYRSPVPSGEMRLAS IAFNHPHIQVDQNLSSYFKLS IPTNFSFGTVQYEYIQT
IQKTVTDCKQVNCFQKEQFLREYGQFSKINQLHAGANLQDDSVRNFLASVYSS

QSSPI IPQFGGDFNLTLLEPVSI STGSRSRSARLIEDLLDFKVTIADPYQGMYQYDCMQ
QPASARLDCIAQVYAGYKVLPPMDVNEAATLSSLGSIAVGWTAGLSSFAAPF
AQSIYFLRNGVTQQLASEFQKLIANKFQALQAMQTGFTTNEAFKVDQAVMNNA
QALSKLASELSNTFGAISASIGDI IQRLDVELQDAQDRIADRLRINGRLTTLANFAQQLVR
SESAAALSAQLADDKVNECVQAKRSGFCQGT GHISVFVNNAPNGLYFMYHVYPSNH

IENVASYGLDAANPTCNIAVPVNGFPIKTNRIVDEWSYTGSFSAPEPETSILNTKY
VAPQVTQSNISTNLPPPLLGNSGTIEIDQFQDELDEFFKNVSTS IPNFSLTQINTTLDL
TYEMLSLQQWKLNESIIDLKELGHNYYNKWPWHYIWGLFIAGLVALACVVFFILCC
TGCNTGKMKLCNCNRCCDRYEYDEPHKVKHVH

Suitably the nucleic acid encoding the spike protein antigen, and/ or encoding the tPA-spike protein antigen fusion, is codon optimised for humans.
Suitably said polynucleotide sequence comprises the sequence of SEQ ID NO: 3

SEQ ID NO: 3 - Without tPA leader

ATGATCCACCTCCGTGTTCCTGCTGATGTTCCTGCTGACCCCCACCGAGAGCTACGTGG
ACGTCGGCCCCGGATAGTCGGAAGTCCGCTGATCGAATGCGACATCCAGACAGGAC

5

TTCCTGCAGAGACCTCCCAAGACCTACAGCTGAGGAGGCTTCTCTGCTGATGCTGG
ACGTGACCTGCTGACAATCGAGGCTGGGTTTCCTCACACCAACCTGGTGTGCTGCTG

TCCACAAGGCAGCGACCTACAGCAACATACACATACATTACCTACACAGGGCCTGTTCC
CAAGGCGACCACGGCGATATGTACGTGTACTCTGCCGGCCACGCCACCGGACACCA

10

CCCCGAAACTGGCTGGCAACTACAAGGGCCAGCCTGGAACCCAGAAGCCAG

GAACGCTGACAACCCGCCTACACGGCCAGCTGAGTCCGCTGCTCCTACGAGAGCTTC

15

GGCACTGAGCTGTCCAGAGCGGGCAAGTGGCGGTTTCTTCAACCAACACCCTGGTGTGCTG

TGACGCGCTGCTGACAATCGAGGCTGGGTTTCCTCACACCAACCTGGTGTGCTGCTG

20

CACCACATCGTCTGGCAGCAGCGACGGGCCATCGACTGCGGCTTCAACGACCTGAGCC

AGCTGCCTGCTCCTACGAGAGCTTCGAGCTGGGAAAGCGGCGTACAGCGGTGCTCAA

25

TCTCGGCAATGCTTGCAGGTTCACCAACCAAGCGGACCGCTGCTGCTTCCGGAAATCG

CTGCTGCTGCTTCCGACGAGGCGGAGCTGGCAATTACTACTGCCTGGCGGCGCTGTG

30

TCCAGATTGGCCGCCCCTTACGTGTAACAAGTGGCAGCCTACCCCTGAGGTGGAATGC

GGCGACTACAGAGACGCTCGAGCGCCCGTACAGCTGAGGAGGCGGACGGGATGCGGCTGG

35

TCTGGAAGCAGATCGCGAGACGGCCATCGAGCAGCTGAGGCTGCTGGCTTGGAAGCA

ATGCGCTGATCGTGCTGAACTACACTGCGGGGAAATCCTCGCTATGCTGGTGGCAG

40

AGATCGGCCAGCTGAGCTGGCCCAAACTGCGTTGGAATATCGCCTGCTGCTGCTG

GGGAGCTGTTCCGCAATGTCACCGAGCTGAGGAGGCGGAGCTGAGAGTCCGCTGCTG

45

ATGCGCTGATCGTGCTGAACTACACTGCGGGGAAATCCTCGCTATGCTGGTGGCAG

AGATCGGCCAGCTGAGCTGGCCCAAACTGCGTTGGAATATCGCCTGCTGCTGCTG

50

GGGAGCTGTTCCGCAATGTCACCGAGCTGAGGAGGCGGAGCTGAGAGTCCGCTGCTG

GGGAGCTGTTCCGCAATGTCACCGAGCTGAGGAGGCGGAGCTGAGAGTCCGCTGCTG

55
More suitably said polynucleotide sequence comprises the sequence of SEQ ID NO: 4 - With tPA leader

ATGGACGCCATGAAGAGGGGCTGCTGTGCTGTGCTGTGCTGTGCGCCGTGTTT
GTGTCGCCAGCCAGAAATCCACGCCCCGTTTCAAGACCGATCCACTCGGCCCAG
TGATGTTTCTGTGACCCCAAGGAGCTACTGGAAGCTGGGACCCAGACGCCGACCTAGTG
AGTCCCGCTTGTATCGAAGTGGGACATCCAGAAGCGCTTTTTTCGCAAGAAGACCCTTG
ACCCATCGACGTTCAAGGCCAGGGAGCTACTGCGGATTGGCGCCGCCCTGCTG
CACACACCAGCTCAGCCACCACCGCCACGCTGCTGCTGCTGCTGCTGCTGGCGCCGC
TGCAATAGCAACCCGACAGTATCATCAGCCACCACAGCGCCCATCCACTCAGCAG
ATCTACCCCGCTTCCATGCTGGGCAAGACGTCGTGCTGGCGGAATTCAGACCGACG
GGGCGGTCTTCCATACCAACCCGTTTGTGCTGTGCTGCTGCTGCTGCTGCTGCTG
GACGCTTTCTACTGCTGGAAACCCAGAAGCCGCAACACTGCTGCTGCTGCTGCTG
CCACACCAGCTTCCAGCCACCACCGCCACCGTATTCTCGACGCAGCACACTAC
AACCCGGAAGCCACGGCTGAAAGACGTCTCAACCTGCTGCTGCTGCTGCTGCTG
TCATGTACACCTAAATTCGAGAGCCAGAATCTTGGGTGCAAGACG
GCCAGCCAGGGCGTCACTGGAGCAGATAGCGCGGAGCTGCTGCTGCTGCTGCTG
GACGCTTGGGAAGCGGCGTGTACAGCGCTACGAGGCACAGCCTAGACGGCGACG
GTGGTGGGGAACAGGTAGGGGTGGAATGAGCTACCTTCCCTGCTGCTGCTGCTG
 CCCACAGCATCCCCGTCAGCTCACAGCGACAGAAGAGCTGGGGCCGCTCTTCTACGTG
ACAAGCTGCAGCCACCTTGACCTCTGGACTCAGGCGTACCTGACCTGACG
GGCATTGCAGCTCGGCTTCAACAGGCACGTGACCTGCTCTGCTGCTGCTGCTG
GAGCTGGGAAAGCGGCGTGTACAGCGCGTCCAAGCATCCAGCGATGCAGCGCGA
AACCCAGGCTGCACTGGCACTCCCTGCTGCTGCTGCTGCTGCTGCTGCTG
CTCTCCAGGTTGACAACTCTCAAGCCGCTGCTGCTGCTGCTGCTGCTGCTG
CCAAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
CCGCAATTGAGACCACTACGGCTAAGAGCGACACGCACGTGCTACGCTGCTGCTG
AGCAGAAGTGGGTAAGGCGGATGCTCTTGGGAGCCAGACCTGCTGCTGCTGCTG
AGCAGAAGTGGGTAAGGCGGATGCTCTTGGGAGCCAGACCTGCTGCTGCTGCTG
CGCCATTTGCGAGCACTGCATGACCCGCTGTGCTGCTGCTGCTGCTGCTGCTG
AGCAGAAGTGGGTAAGGCGGATGCTCTTGGGAGCCAGACCTGCTGCTGCTGCTG
CGCCATTTGCGAGCACTGCATGACCCGCTGTGCTGCTGCTGCTGCTGCTGCTG
AGCAGAAGTGGGTAAGGCGGATGCTCTTGGGAGCCAGACCTGCTGCTGCTGCTG
CGCCATTTGCGAGCACTGCATGACCCGCTGTGCTGCTGCTGCTGCTGCTGCTG
Suitably administration of a single dose of said composition to a mammalian subject induces protective immunity in said subject.

In one aspect, the invention relates to a composition as described above for induction of an immune response against MERS-CoV.

In one aspect, the invention relates to a composition as described above for induction of an immune response against MERS-CoV, wherein a single dose of said composition is administered.

In one aspect, the invention relates to a composition as described above for induction of an immune response against MERS-CoV, wherein said composition is administered once. Suitably said composition is administered once per 6 months. Suitably said composition is administered once per 12 months.

Suitably the primary vaccination regimen is one dose, whereas all prior art MERS vaccines require two doses to provide protection. In some embodiments it may be desired to re-administer at a later date, not less than 6 months after the first immunisation.

In one aspect, the invention relates to a composition as described above for preventing MERS-CoV infection.

In one aspect, the invention relates to a composition as described above for preventing MERS-CoV infection, wherein a single dose of said composition is administered.

In one aspect, the invention relates to a composition as described above for preventing MERS-CoV infection, wherein said composition is administered once. Suitably said composition is administered once per 6 months. Suitably said composition is administered once per 12 months.

In one aspect, the invention relates to use of a composition as described above in medicine.

In one aspect, the invention relates to use of a composition as described above in the preparation of a medicament for prevention of MERS-CoV infection.
In another aspect, the invention relates to use of a composition as described above in inducing an immune response against middle eastern respiratory syndrome coronavirus (MERS-CoV). In another aspect, the invention relates to use of a composition as described above in immunising a subject against MERS-CoV. In another aspect, the invention relates to use of a composition as described above in preventing MERS-CoV infection.

A method of inducing an immune response against middle eastern respiratory syndrome coronavirus (MERS-CoV) in a mammalian subject, the method comprising administering a composition as described above to said subject.

Suitably a single dose of said composition is administered to said subject. Suitably said composition is administered once. Suitably said composition is administered once per 6 months. Suitably said composition is administered once per 12 months.

Suitably said composition is administered by a route of administration selected from a group consisting of subcutaneous, intranasal, aerosol, nebuliser, intradermal and intramuscular.

Most suitably said administration is intramuscular.

In one aspect the invention relates to an adeno-based viral vector comprising nucleic acid having a polynucleotide sequence encoding the spike protein from the middle eastern respiratory syndrome coronavirus (MERS-CoV). Suitably the adeno-based viral vector is ChAdOx 1.

In one aspect, the invention relates to a ChAdOx comprising a polynucleotide encoding glycoprotein S from the MERS virus. Suitably said adeno-based viral vector has the sequence and/or construction as described in one or more of the examples.

In one aspect, the invention relates to a method of raising an immune response by administering the adeno-based viral vector as described above.

In one aspect, the invention relates to the adeno-based viral vector as described above for use in preventing MERS infection.

In one aspect, the invention relates to the adeno-based viral vector as described above for use in raising an anti-MERS immune response.

**DETAILED DESCRIPTION**
It is believed that the combination of ChAdOxi with the MERS antigen used in this work has not been disclosed previously and is therefore novel.

It is believed that the prior art suggests a prejudice against the use of MERS antigens to develop a vaccine - Hotez (ibid.) has identified a risk of MERS vaccines based on S protein as possibly being affected by the risk of inducing eosinophilic hypersensitivity which was seen with S protein based SARS vaccine. Hypersensitivity in mice with MERS vaccines from the same laboratory has been noted. It is an advantage of the invention that no such hypersensitivity is seen.

Prior art prime-boost using MVA-based vaccine candidates produces a very robust immune response as has been demonstrated repeatedly with a large number of different antigens in various indications. It is an advantage of the invention that one administration of ChAdOxi-MERS raised an immune response equivalent to that of a homologous MVA prime-boost regimen against the same antigen. This comparable response from a single dose of ChAdOxi-MERS was unexpected and has a number of benefits including quicker, simpler treatment and cheaper manufacturing and treatment.

We disclose that ChAdOxi and MVA-based vaccine candidates against MERS-CoV elicit neutralising antibodies and cellular immune responses in mice. We describe four vaccines against MERS-CoV based on ChAdOxi and MVA viral vectors, two vaccines per vector. All vaccines contained the full-length spike gene of MERS-CoV; ChAdOxi MERS vaccines were produced with or without the leader sequence of the human tissue plasminogen activator gene (tPA) where MVAMERS vaccines were produced with tPA, and either the nM5 or F11 promoter driving expression of the spike gene. All vaccine candidates were evaluated in a mouse model in prime only or prime-boost regimens. ChAdOxi MERS with tPA induced higher neutralising antibodies than ChAdOxi MERS without tPA. A single dose of ChAdOxi MERS with tPA elicited cellular immune responses as well as neutralising antibodies - these were boosted to a significantly higher level by MVAMERS. The humoral immunogenicity of a single dose of ChAdOxi MERS with tPA was equivalent to two doses of MVAMERS (also with tPA). MVAMERS with nM5 or F11 promoter induced similar antibody levels. The F11 promoter enhanced the cellular immunogenicity of MVAMERS to significantly higher magnitudes. In conclusion, our study showed that MERS-CoV vaccines could be optimised by utilising different viral vectors, and/or
various genetic designs of the vectors, and/or different regimens to increase immunogenicity. ChAdOxi and MVA vectored vaccines have been safely evaluated in camels and humans and these MERS vaccine candidates are demonstrated to be promising and the data shown herein support their industrial application for example that they are expected to be effective in camels and in clinical trials in humans.

We show development of MERS-CoV vaccine candidates that are based on two different viral vectors: Chimpanzee Adenovirus, Oxford University #1 (ChAdOxi) (26) and Modified Vaccinia virus Ankara (MVA) (27, 28). Each viral vector was developed by generating two alternative versions, resulting in four vaccine candidates that all encode the same complete MERS-CoV spike gene (S). The two ChAdOxi based vaccines were produced with or without the signal peptide of the human tissue plasminogen activator gene (tPA) at the N terminus. Previous studies have shown that encoding tPA upstream of recombinant antigens enhanced immunogenicity, although results differed depending on the antigens employed. The tPA encoded upstream of influenza A virus nucleoprotein, in a DNA vector, enhanced both cellular and humoral immune responses in mice (29, 30), whereas the same leader sequence resulted in increased humoral sequences but decreased cellular responses to fHIV Gag (30). The two MVA based vaccines were produced with either the mH5 or F11 poxviral promoter driving antigen expression, both including the tPA sequence at the N terminus of MERS-CoV Spike protein. Previously, we reported the ability of the strong early F11 promoter to enhance cellular immunogenicity of vaccine antigen candidates for malaria and influenza, as compared to utilising P7.5 or mH5 early/late promoters which resulted in a lower level of gene expression immediately after virus infection of target cells, but higher levels at a later stage (31). Here, we continue to assess the F11 promoter in enhancing cellular immunogenicity, and to investigate its ability to impact on humoral immune responses. The four vaccine candidates were evaluated in a number of different regimens in mouse models that showed a single dose of ChAdOxi MERS inducing higher cellular and humoral immunogenicity than a single dose of MVA MERS, or equivalent to two doses of MVAMERS. ChAdOxi based vaccines have been tested in different animal models, including camels (32), and in human clinical trials and proved safe and immunogenic (33). Therefore, based on our data, ChAdOxi MERS can be readily employed for use as a MERS vaccine in humans. Furthermore, utilising ChAdOxi MERS for camel vaccination can serve the one-health approach whereby blocking MERS-CoV transmission in camels is expected to prevent human infections.
The inventors identified the major surface antigen of MERS-CoV as the Spike (S protein) and demonstrated that ChAdOxi expressing this protein induces the production of anti-S antibodies, including neutralising antibodies, after a single intramuscular immunisation. This single dose induced a response equivalent to that provided by a prime-boost administration of the same antigen in another viral vector as demonstrated in mice. Studies in camels and humans are outlined below. Hypersensitivity responses were not seen in vaccinated animals and human subjects.

APPLICATIONS

The invention finds particular application in prevention or containment of outbreaks of MERS. In this scenario, it is extremely advantageous to achieve protective immunity with only a single dose of vaccine. In the special considerations which apply to emerging pathogens such as MERS, there is typically not time to give two doses. It is also exceptionally difficult to recall patients for their second dose. For example, patients may need to walk from village to village in order to receive their dose and have many pressures on their time which can prevent attendance for a second dose. For example, they may have to travel from distance to receive a dose, or they may need to attend to their livelihoods which can prevent them from attending for more than a single dose. Thus, there is a need for a rapid onset of protection, which need is met by the present invention. The present invention also advantageously allows for avoidance of quarantine of patients in between doses which might otherwise be required since acquiring the infection in between doses would be potentially deleterious for the individual.

The invention finds particular application in the immunisation of camels. Camels are believed to be the only, or at least the main, animal source of human infection with MERS. For similar reasons as outlined above, it is a technical benefit that the invention delivers protective immunity with only a single dose - this advantage extends to camels as well as to human or other subjects.

Suitably the subject is a camel.

Suitably the subject is a human.

As acknowledged in the background to the invention, Malczyk et al. 2015 disclose a measles virus vaccine comprising the MERS spike protein. However, protective immunity is only demonstrated for this measles based vaccine after two doses. Thus, the invention delivers benefits compared to Malczyk et al. 2015.
Suitably the method is a method of immunising.

Suitably the immune response comprises a humoral response. Suitably the immune response comprises an antibody response. Suitably the immune response comprises a neutralising antibody response. Suitably the immune response comprises a cell mediated response. Suitably the immune response comprises cell mediated immunity (CMI). Suitably the immune response comprises induction of CD8+ T cells. Suitably the immune response comprises induction of a CD8+ cytotoxic T cell (CTL) response. Suitably the immune response comprises both a humoral response and a cell mediated response. Suitably the immune response comprises protective immunity.

Suitably the composition is an antigenic composition.

Suitably the composition is an immunogenic composition.

Suitably the composition is a vaccine composition.

Suitably the composition is a pharmaceutical composition.

Suitably the composition is formulated for administration to mammals, suitably to primates or camelids. Camelids are members of the biological family *Camelidae*, the only currently living family in the suborder *Tylopoda*. The extant members of this group are: dromedary camel, Bactrian camels, wild or feral camels, llamas, alpacas, vicunas, and guanacos. More suitably the composition is formulated for administration to primates, camels, alpacas, llamas, vicunas, pigs, non-human primates or humans, more suitably to primates or camels, most suitably to humans.

It may be useful to protect pigs against MERS. The virus does productively infect pigs (does not apply to sheep, cattle, chickens). Although there are few farmed pigs in the middle east, there are wild boars which may get infected and form another zoonotic reservoir which may then spread, or if MERS gets into farmed pigs somewhere else in the world, such as US or China, it would be a major problem. Thus the invention finds utility in this application.
Suitably the composition is formulated taking into account its route of administration. Suitably the composition is formulated to be suitable for the route of administration specified. Suitably the composition is formulated to be suitable for the route of administration selected by the operator or physician.

DATABASE RELEASE

Sequences deposited in databases can change over time. Suitably the current version of sequence database(s) are relied upon. Alternatively, the release in force at the date of filing is relied upon.

As the skilled person knows, the accession numbers may be version/dated accession numbers. The citeable accession numbers for the current database entry are the same as above, but omitting the decimal point and any subsequent digits.

GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA; Nucleic Acids Research, 2013 Jan;4i(Di):D36-42) and accession numbers provided relate to this unless otherwise apparent. Suitably the GenBank database release referred to is 15 April 2017, NCBI-GenBank Release 219.0.

UniProt (Universal Protein Resource) is a comprehensive catalogue of information on proteins (‘UniProt: a hub for protein information’ Nucleic Acids Res. 43: D204-D212 (2015).). For the avoidance of doubt, UniProt Release 20i5_n is relied upon.

In more detail, the UniProt consortium European Bioinformatics Institute (EBI), SIB Swiss Institute of Bioinformatics and Protein Information Resource (PIR)'s UniProt Knowledgebase (UniProtKB) Release 20i7_05, (io-May-2017) is relied upon.

ADVANTAGES

The inventors have previously published a paper immunising against River Valley Fever (RVF). In this paper, protective immunity is achieved after only one dose of ChAdOxi containing the RVF antigen. However, RVF is not a coronavirus. RVF is a completely different type of virus. Thus, it would not be correct to extrapolate from the RVF study as if it could be expected that a single dose might produce protective
immunity in other viral families. It is an advantage of the invention that a single dose delivers protective immunity against MERS.

Weingartl et al. 2004 (ibid) discloses the use of a MVA vector to deliver antigen to ferrets. However, Weingartl et al. observed pathology using this viral delivery system. Therefore, it would be incorrect to extrapolate from this paper as if merely using a viral vector would be the answer to achieving single dose immunity.

It should be noted that numerous publications (as explained in the background to the invention above) have attempted to use diverse viral vectors to deliver the MERS spike protein as an antigen for vaccination, but those prior art approaches have each suffered from immunopathology (sometimes described as hypersensitivity) effects such as eosinophil infiltration of the lungs. These very serious medical complications are advantageously overcome by the present invention.

This view in the art is further established by the Hotez review (ibid) which noted the numerous problems causing immunopathology in attempting to immunise against the SARS coronavirus. The Hotez review clearly identifies this as a problem extending to MERS vaccines. Advantageously, the present invention delivers a solution to this problem.

Thus, the invention delivers the advantage of avoiding immunopathology.

The invention provides the advantage of avoiding hypersensitivity.

The invention possesses the advantage of protective immunity after single dose (single administration).

The phrase "protective immune response" or "protective immunity" as used herein means that the composition is capable of generating a protective response in a host organism, such as a human or a non-human mammal (e.g. a camel), to whom it is administered according to the invention. Suitably a protective immune response protects against subsequent infection or disease caused by MERS-CoV.

These advantages flow from the particular combination of features as set out in the claims.
SPIKE PROTEIN

Suitably the antigen in the MERS spike protein.

Suitably the full length spike protein is used.

Suitably full length means each amino acid in the spike protein is included.

An exemplary spike protein is as disclosed in accession number AHX71946.1 - SEQ ID NO: 1 (above).

It may be possible to use only the Si domain of the spike protein, or only the soluble part of the spike protein, or only the receptor binding domain of the spike protein. However, most suitably according to the present invention the full length spike protein is used.

By choosing the full length spike protein, advantageously the correct confirmation of the protein in assured. Truncated proteins can assume unnatural conformations. This drawback is avoided by using the full length protein.

A further advantage of using the full length spike protein is that it allows for better T-cell responses. Without wishing to be bound by theory, it is believed that the more amino acid sequences present, then the more potential targets there are for the T-cell responses. Thus, suitably every amino acid of the wild type spike protein is included in the antigen of the invention.

tPA

tPA (tissue plasminogen activator) - more specifically the tPA leader sequence - is suitably fused to the MERS spike protein antigen of the invention. Suitably tPA is fused to the N-terminus of the spike protein sequence.

Suitably tPA leader sequence means the tPA amino acid sequence of SEQ ID NO: 5

SEQ ID NO: 5

MDAMKRGLCCVLLLCGAVFVSASQEIHARFRR
In the above SEQ ID NO: 5 the C terminal 'RR' is not actually part of the tPA leader sequence. It comes from the fusion of two restriction sites. Suitably the tPA leader sequence may be used with or without the C terminal 'RR' e.g. SEQ ID NO: 7 or SEQ ID NO: 8. Most suitably the sequence is used as shown in SEQ ID NO: 5.

The underlined A is P in the naturally occurring tPA leader sequence. The P->A mutation has the advantage of improved antigen secretion. Suitably the tPA leader sequence may be used with or without the P->A mutation, i.e. suitably the tPA leader sequence maybe used as SEQ ID NO: 5 or SEQ ID NO: 6.

SEQ ID NO: 5
MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR

SEQ ID NO: 7 (=SEQ ID NO: 5 without C-terminal 'RR')
MDAMKRGLCCVLLLCGAVFVSASQEIHARF

SEQ ID NO: 8 (=SEQ ID NO: 6 without C-terminal 'RR')
MDAMKRGLCCVLLLCGAVFVSPSQEIHARF

More suitably the sequence is used with the P->A mutation (with or without the C terminal 'RR'). Most suitably the sequence is used as shown in SEQ ID NO: 5.

An exemplary nucleotide sequence encoding tPA, which has been codon optimised for human codon usage, is as shown in SEQ ID NO: 9 (this is the sequence encoding SEQ ID NO: 5):
ATGGACGCCATGAAGAGGGGCCTGTGCTGCGTGCTGCTGCTGTGGCGCCGTGTTTGTTGTCCCGCCAGCCAGGAAATCCACGCCCCGGTTCAGACGG

It is believed that tPA promotes secretion of proteins to which it is fused. It is believed that tPA increases expression of proteins to which it is fused. Notwithstanding the underlying mechanism, the advantage in the invention of fusing tPA to the N-terminus of the spike protein antigen is that improved immunogenicity is achieved. Thus, most suitably the antigen of the invention is provided as a fusion with tPA. Most suitably the tPA is fused to the N-terminus of the spike protein antigen.

Suitably the antigen does not comprise any further sequence tags.

Suitably the antigen does not comprise any further linker sequences.

Adeno-based viral vectors
Any suitable adeno-based viral vector may be used.

In more detail, any replication-deficient viral vector, for human use preferably derived from a non-human adenovirus may be used. For veterinary use Ad5 may be used.

ChAdOx2 is an example of a suitable non-human adenovirus vector for human use.

Most suitably the adeno-based viral vector is ChAdOxi.

ChAdOxi


For insertion of the nucleotide sequence encoding spike protein, suitably the E1 site may be used, suitably with the hCMV IE promoter. Suitably the short or the long version may be used; most suitably the long version as described in WO2008/122811, which is specifically incorporated herein by reference for the teaching of the promoters, particularly the long promoter.

It is also possible to insert antigens at the E3 site, or close to the inverted terminal repeat sequences, if desired.

In addition, a clone of ChAdOxi containing GFP is deposited with the ECACC: a sample of *E. coli* strain SW1029 (a derivative of DH10B) containing bacterial artificial chromosomes (BACs) containing the cloned genome of AdChOXi (pBACe3.6 AdChOxi (E4 modified) TIPeGFP, cell line name "AdChOxi (E4 modified) TIPeGFP") was deposited by Isis Innovation Limited on 24 May 2012 with the European Collection of Cell Cultures (ECACC) at the Health Protection Agency Culture Collections, Health Protection Agency, Porton Down, Salisbury SP4 0JG, United Kingdom under the Budapest Treaty and designated by provisional accession no. 12052403. Isis Innovation Limited is the former name of the proprietor/applicant of this patent/application.
ChAd0x2

The nucleotide sequence of the ChAd0x2 vector (with a Gateway™ cassette in the El locus) is shown in SEQ ID NO. 2. This is a viral vector based on Chimpanzee adenovirus C68. (This is the sequence of SEQ ID NO: 10 in gb patent application number 1610967.0).

In addition, a clone of ChAd0x2 containing GFP is deposited with the ECACC: deposit accession number 16061301 was deposited by Isis Innovation Limited on 13 June 2016 with the European Collection of Cell Cultures (ECACC) at the Health Protection Agency Culture Collections, Health Protection Agency, Porton Down, Salisbury SP4 0JG, United Kingdom under the Budapest Treaty. Isis Innovation Limited is the former name of the proprietor/applicant of this patent/application.

ADMINISTRATION ROUTE

In principle any suitable route of administration may be used.

Suitably the route of administration is selected from group consisting of subcutaneous, intranasal, aerosol, nebuliser, intradermal and intramuscular.

Suitably the route of administration is selected from a group consisting of intranasal, aerosol and intramuscular.

Suitably the route of administration is selected from a group consisting of intranasal and intramuscular.

Most suitably the route of administration is intramuscular.

The route of administration may be applied to camels or humans.

DOSE

It should be noted that there are alternate ways of describing the dose for adenoviral vectors.
• Viral particles - vp/mL. This refers to the count of total viral particles administered.
• Infectious units - i.u./mL. This refers to the number of infectious units administered, and can be correlated more accurately with immunogenicity.

By convention, clinical trials in the UK tend to provide the dose in terms of viral particles.

Preferred doses according to the present invention are:

Humans, preferred range is from \(10^9\) to \(10^{11}\) viral particles
Camels, \(10^9\) to \(10^{12}\) viral particles
Pigs, \(10^8\) to \(10^{10}\) viral particles.

Suitably no adjuvant is administered with the viral vector of the invention.

Suitably the viral vector of the invention is formulated with simple buffer. An exemplary buffer may be as shown below under the heading 'Formulation'.

**FURTHER FEATURES**

Suitably the nucleic acid sequence is codon optimised for mammalian codon usage, suitably for camel or human codon usage, most suitably for human codon usage.

Suitably a container containing a composition as described above is provided. Suitably said container may be a vial. Suitably said container may be a syringe.

Suitably a nebuliser containing a composition as described above is provided.
Suitably a nasal applicator containing a composition as described above is provided.
Suitably an inhaler containing a composition as described above is provided.

Suitably a pressurised canister containing a composition as described above is provided.

A method of making a composition as described above is provided, said method comprising preparing a nucleic acid encoding the MERS-CoV spike protein, optionally fused to the tPA protein, and incorporating said nucleic acid into an adeno-based viral vector, suitably a ChAdOxi vector. Suitably the nucleic acid is operably linked to a promoter suitable for inducing expression of said MERS-CoV spike protein (or MERS-
CoV spike protein-tPA fusion protein) when in a mammalian cell such as a human or camel cell.

**FORMULATION**

The ChAdOxi formulation buffer, as used for the clinical product is:

**FORMULATION BUFFER COMPONENTS**

1. 10 mM Histidine
2. 7.5% Sucrose
3. 35 mM Sodium chloride
4. 1 mM Magnesium chloride
5. 0.1% Polysorbate 80
6. 0.1 mM EDTA
7. 0.5% Ethanol
8. Hydrochloric acid (for pH adjustment to ~pH 6.6)

Formulated in Water for Injection Ph Eur.

Formulations for other administration routes such as aerosol will be adjusted accordingly by the skilled operator.

Suitably the composition and/or formulation does not comprise adjuvant. Suitably adjuvant is omitted from the composition and/or formulation of the invention.

**MVA- MERS SPIKE PROTEIN**

An MVA-MERS spike protein vaccine has been described in the prior art, for example by Volz et al. 2015/Haagmans et al. 2016 (ibid). We also disclose an MVA vector carrying the MERS spike protein, which is described in more detail in the Examples section. The MVA vector described herein features a \( \text{N}_{11}/\text{F11} \) promoter system in one embodiment, or relies on a standard F11 promoter in another embodiment. In any case, these promoter systems are known in the art, for example in published patent US 9,273,327B2 (Cottingham - granted 1 March 2016 - 'Poxvirus Expression System') - this document is hereby incorporated by reference, in particular for the specific teachings of promoter(s) for use herein.
In the context of the present invention, MVA vector delivering MERS spike protein is taught as a useful optional boost in an immunisation regimen as described. The first dose should preferably be ChAdOxi-MERS spike protein (most preferably comprising the tPA fusion to the N-terminus of the spike protein) and the optional second administration preferably comprises MVA-MERS spike protein.

As will be apparent, the main focus of the invention is in provision of a single dose MERS vaccine. However, in this embodiment featuring a second (boosting) administration, preferably the second (boosting) administration is in a different viral vector i.e. a heterologous "prime-boost" regime. Suitably the second (boosting) administration comprises a MVA vector. This finds particular application for example in inducing immunity in subjects such as healthcare workers. It is a particular problem that healthcare workers can contract a MERS infection. Since they are typically in good health themselves, this has very little effect, if any, on their general health. However, when they are infected they can of course excrete virus, which can go on to infect immune compromised patients in their care with disastrous consequences. Therefore, there is a special and particular problem in the immunisation of healthcare workers. A durable and long lasting immunity is desired for these professionals. Therefore, whilst it is a core tenet of the invention that a single dose of vaccine provides protection against MERS infection, in the special case of healthcare workers the protective immunity is desired to last as far as possible into the future. For most applications, a temporary immunity ("temporary" contrasted with a lifelong immunity) is entirely adequate to protect the individual and/or to halt the spread of the infection. However, in the special case of healthcare workers any way of extending their immunity in time is itself additionally advantageous. In this scenario, we teach a "prime-boost" regimen comprising a first administration of an adenoviral vector-MERS composition such as a ChAdOx-MERS composition, followed by a second (boosting) administration of a viral vector comprising the MERS spike protein, such as a MVA vector expressing the MERS spike protein. Thus, in the inventors' opinion, MVA-MERS spike protein has limited use but may find particular application as a heterologous boost following a ChAdOx-MERS spike protein priming vaccination. In one embodiment the order of immunisations may be reversed so that the MVA-MERS vaccine is administered first followed by the ChAdOx-MERS vaccine after an interval of typically 1-8 weeks.

Similarly, MVA boosting for extended duration of immunity may also be useful for subjects who are occupationally exposed to camels, e.g. camel shepherds or slaughterhouse workers. They may also become infected, experience only mild illness
but transmit the infection to family or community members. There is a slightly higher rate of seropositivity to MERS in these workers than the general population. Thus this embodiment of the invention may be applied to such subjects who are occupationally exposed to camels, most suitably camel shepherds or slaughterhouse workers.

Thus in one aspect the invention provides a method of inducing an immune response against middle eastern respiratory syndrome coronavirus (MERS-CoV) in a mammalian subject, the method comprising

(i) administering a composition comprising a viral vector, the viral vector comprising nucleic acid having a polynucleotide sequence encoding the spike protein from the middle eastern respiratory syndrome coronavirus (MERS-CoV), characterised in that said viral vector is an adenovirus based vector to said subject, and

(ii) administering a composition comprising a viral vector, the viral vector comprising nucleic acid having a polynucleotide sequence encoding the spike protein from the middle eastern respiratory syndrome coronavirus (MERS-CoV), characterised in that said viral vector is a MVA based vector to said subject.

Suitably step (i) is a priming composition.

Suitably step (ii) is a boosting composition.

Suitably step (ii) is carried out 1-8 weeks after the step (i), most suitably 4 weeks after step (i).

Further particular and preferred aspects are set out in the accompanying independent and dependent claims. Features of the dependent claims may be combined with features of the independent claims as appropriate, and in combinations other than those explicitly set out in the claims.

Where an apparatus feature is described as being operable to provide a function, it will be appreciated that this includes an apparatus feature which provides that function or which is adapted or configured to provide that function.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will now be described by way of example, with reference to the accompanying drawings, in which:

**Figure 1: Construction of MERS-CoV vaccine candidates**

A schematic representation of ChAdOxi and MVA based vaccines, each encodes the same MERS-CoV spike gene (MERS-CoV S). The S gene was inserted into the E1 region
of ChAdOxi genome or into the F11L locus of MVA genome. tPA: Human tissue plasminogen activator (tPA) signal peptide sequence. IE CMV: The human cytomegalovirus major immediate early promoter. ηH5 and F11: Poxviral promoters. LHA: left homology arm sequence. RHA: right homology arm sequence. B: The expression of spike transgene, cloned into a plasmid vector, was validated by transfection into an African green monkey kidney cell line (Vero cells) and confirmed by immunostaining. C: Untransfected cells control. Green colour represents detection of the spike protein. Blue colour represents nuclei by staining nucleic acid with DAPI.

**Figure 2: Antibody responses to ChAdOxi MERS vaccine candidates.**

BALB/c mice (n = 6) were immunised with a single injection of ChAdOxi MERS that either encodes or lacks tPA signal peptide, intramuscularly at ixio ^8^ IU. A control group of mice were immunised with ChAdOxi expressing eGFP instead of MERS-CoV S gene. Serum samples were collected at 14 and 28 days post immunisation (d.p.i.). Si-binding antibodies were detected at both time points by ELISA (A) and neutralisation activity of the antibodies were confirmed by MERS-CoV pseudotyped viral particles (MERSpp) neutralisation assay (B) or neutralisation assay (C). Individual data points are shown with line as the median. Data are representative of two independent experiments. Statistical significance by Kruskal-Wallis test is shown.

**Figure 3: Cellular immune responses to ChAdOxi MERS vaccine candidate.**

BALB/c mice (n = 6) were immunised with a single injection of ChAdOxi MERS that encodes tPA signal peptide intramuscularly at ixio ^8^ IU. Twenty eight days post-immunisation, IFN-γ ex vivo ELISpot (A) or Intracellular Cytokine Staining (ICS (B)), were performed to determine the percentage of splenic IFN-γ secreting CD4^+^ and CD8^+^ after in vitro re-stimulation with a MERS-CoV S-specific peptide. Individual data points are shown with line as the median (A) or error bars as the SD (B). Data are representative of two independent experiments.

**Figure 4: Humoral and cellular immunogenicity of heterologous ChAdOxi MERS and MVA MERS vaccination.**

BALB/c mice (n = 6) were immunised with ChAdOxi MERS that encodes tPA signal peptide, intramuscularly at ixio ^8^ IU. At 28 d.p.i. mice were boosted with MVAMERS at ixio ^6^ pfu. MVAMERS candidates either contain ηH5 or F11 promoter for transgene expression. Serum samples were collected at 28 (post-prime) and 42 (post-boost) d.p.i. Si-binding antibodies were detected at both time points by ELISA (A) and neutralisation activity of serum antibodies at 42 d.p.i. were confirmed by MERSpp neutralisation assay (B). At 42 d.p.i, IFN-γ ex vivo ELISpot (C) or Intracellular Cytokine Staining (ICS (D)) were performed to determine the percentage of CD8^+^ IFN-γ splenocytes after in vitro re-stimulation with a MERS-CoV S-specific peptide. ICS of
splenocytes re-stimulated with MVA-specific peptides (F(G)2 and E3) was also performed (E and F). Individual data points are shown with line as the median. Data are representative of two independent experiments. Statistical significance by Kruskal-Wallis test is shown. Symbols are closed squares (∗) for ChAdOxi prime responses, open circles (○) for 1T1H5-MVA boost responses, and closed circles (·) for F11-MVA boost responses.

**Figure 5:** Humoral and cellular immunogenicity of homologous MVA MERS vaccination.

BALB/c mice (n = 6) were immunised with MVAMERS at ixio A^6 pfu, intramuscularly, in a homologous prime-boost vaccination with three-weeks interval. MVAMERS candidates either contain τH5 or F11 promoter for transgene expression. Serum samples were collected at 21 (post-prime) and 42 (post-boost) d.p.i. Si-binding antibodies were detected at both time points by ELISA (A) and neutralisation activity of serum antibodies at 42 d.p.i. were confirmed by MERSpp neutralisation assay (B). At 42 d.p.i splenocytes were processed and re-stimulated with a MERS-CoV S-specific peptide (CD8^+ T cell specific) for IFN-γ ex vivo ELISpot (C). ICS of splenocytes re-stimulated with MVA-specific peptides (F(G)2 and E3) was also performed (D and E) as was performed in figure 4. Individual data points are shown with line as the median. Data are representative of two independent experiments. Statistical significance by Kruskal-Wallis test is shown. Symbols are open circles (○) for 1T1H5-MVA and closed circles (·) for F11-MVA.

**Figure 6. Protective Efficacy of ChAdOxi MERS vaccine.** Groups of 10 mice were vaccinated with 10^8 TCID_{50} ChAdOxi GFP or ChAdOxi MERS via the intranasal or intramuscular route, blood samples were collected before vaccination, and before challenge at 28 days post vaccination. hDPP4 mice were challenged intranasally with 10^4 TCID_{50} MERS-CoV (strain HC0V-EMC2012). At three dpi, four animals were sacrificed and lungs collected for analyses. The remaining six animals per group were sacrificed 28 dpi, or when they reached the endpoint criteria. A) Neutralizing antibody titers of hDPP4 mouse serum samples against MERS-CoV strain HC0V-EMC/2012 after vaccination. B) Weight loss after intranasal challenge with 10^4 TCID_{50} MERS-CoV. After challenge mice were weighed daily and percent body weight per group was calculated compared to body weight at the time of challenge. C) Survival curves of the vaccinated groups. After challenge hDPP4 mice were sacrificed due to the severity of disease signs or at 28 dpi. D) MERS-CoV viral loads in the lower respiratory tract of vaccinated hDPP4 mice. Viral load in the lungs of hDPP4 mice at 3 dpi. Mean values ± SD were calculated. Statistical significance was calculated using the Mann-Whitney U test; p-values: * < 0.05. (D). All experimental procedures were performed as previously
described. Red = ChAdOxi GFP intranasally vaccinated animals; Grey = ChAdOxi MERS intranasally vaccinated animals; Blue = ChAdOxi GFP intramuscularly vaccinated animals; Purple = ChAdOxi MERS intramuscularly vaccinated animals.

Figure 7. Immunohistochemistry staining for MERS-CoV antigen in respiratory tract of vaccinated hDPP4 mice. hDPP4 mouse tissues were evaluated for pathology and the presence of viral antigen as described previously. Briefly, tissues were fixed in 10% neutral-buffered formalin for 7 days and paraffin-embedded. Tissue sections were stained with hematoxylin and eosin (H&E). An in-house produced rabbit polyclonal antiserum against HCoV-EMC/2012 (1:1000) was used as a primary antibody for the detection of viral antigen. Grading of histopathology and immunohistochemistry was done blinded by a board-certified veterinary pathologist. Lung tissues are shown at 100X and 1000X (insert) magnification. ChAdOxi GFP intranasally (A) and intramuscularly (B) vaccinated animals. Lung tissue shows multifocal scattered positivity in the lungs. The inserts display MERS-CoV antigen within the Type I & II pneumocytes. ChAdOxi MERS intranasally (C) and intramuscularly (D) vaccinated animals. No MERS-CoV antigen positivity observed.

Figure 8 shows a plot.

Figure 9 shows a plot, a bar chart and a graph.

Figure 10 shows photographs.

Figure 11 shows a plot of ELISPOT responses to ChAdOxi MERS vaccine in Group 1 (5 x 10^9 vp, low dose) volunteers.

Figure 12 shows a plot of evaluation of MERS-CoV specific antibodies in camels at different ages using the commercial ELISA from Euroimmun.

Figure 13 shows a plot of evaluation of MERS-CoV specific antibodies in camels at different ages using an in house endpoint titre ELISA.

Figure 14 shows a plot of virus neutralisation titres for control and vaccinated camels.

Figure 15 shows a plot of naïve camels: assessment of MERS-CoV specific antibodies in vaccinated camel sera using Euroimmun ELISA kit.

Figure 16 shows a plot of seropositive camels: assessment of MERS-CoV specific antibodies in vaccinated camel sera using Euroimmun ELISA kit.

Figure 17 shows naïve camels: assessment of MERS-CoV specific antibodies in vaccinated camel sera using an in house end point titre ELISA.

Figure 18 shows a plot of seropositive camels: assessment of MERS-CoV specific antibodies in vaccinated camel sera using an in house end point titre ELISA.
EXAMPLES

Materials and methods

Transgene and shuttle vector cloning

The spike (S) gene of MERS-CoV camel isolate (Genbank accession number: KJ650098.1) was synthesised by GeneArt Gene Synthesis (Thermo Fisher Scientific). The S transgene was then cloned into four shuttle plasmid vectors following In-Fusion cloning (Clontech). Two plasmids contained the S transgene within the E1 homologous region of ChAdOxi, driven by the human cytomegalovirus major immediate early promoter (IE CMV) that includes intron A. One of the ChAdOxi shuttle plasmids was designed to include the tPA signal sequence upstream of the transgene sequence while the second plasmid did not contain the tPA. The ChAdOxi shuttle plasmids contained the S transgene within Gateway® recombination cassettes. To construct MVAMERS, one of the shuttle plasmids for MVA was designed to have the upstream and downstream (flanks) of the F11L ORF as homologous sequence arms. Inserting the S transgene within these arms enabled the utilisation of the endogenous F11 promoter, which is part of the right homologous arm, while deleting the native F11L ORF. This resulted in the shuttle vector for generation of Fii-MVA MERS (F11 shuttle vector). The mH5 promoter sequence was subcloned upstream of the S transgene; and this mH5-S transgene was then subcloned into the F11 shuttle vector. This resulted in the shuttle vector for generation of mH5-MVA MERS (Fn/mH5 shuttle vector). mH5-MVA MERS contained the mH5 promoter at the F11L locus, however, the endogenous F11 promoter is intact and located upstream of the mH5 promoter. The endogenous F11 promoter could not be replaced with the mH5 since it is part of the essential upstream ORF.

Immunostaining for Transgene Expression

The ChAdOxi shuttle plasmid, described above, was used to validate the expression of MERS-CoV spike protein in vitro. An African green monkey kidney cell line (Vero cells) was seeded into 6-well plate to 80% confluence. Then the plasmid DNA was transfected into Vero cells using Lipofectamine® 2000 (Thermo Fisher Scientific) following manufacturer’s instruction. Twenty four hours after transfection, cells were fixed, permeabilised, and immunostained using a rabbit polyclonal anti-MERS-CoV spike antibody, following standard protocols. DAPI stain was used to label nuclei.

Construction of recombinant ChAdOxi and MVA encoding MERS-CoV S antigens

The ChAdOxi MERS vaccines were prepared by Gateway® recombination between the ChAdOxi destination DNA BAC vector (described in (26)) and entry plasmids containing the coding sequence for MERS-CoV spike gene (ChAdOxi shuttle vectors
explained above), according to standard protocols. ChAdOxi MERS genomes were then derived in HEK293A cell lines (Invitrogen, Cat. R705-07), the resultant viruses were purified by CsCl gradient ultracentrifugation as previously described (34). The titres were determined on HEK293A cells using anti-hexon immunostaining assay based on the QuickTiter™ Adenovirus Titer Immunoassay kit (Cell Biolabs Inc). For MVAMERS vaccines chicken embryo fibroblast cells (CEFs) were infected with MVA parental virus that encodes dsRed marker instead of the native F11L ORF and transfected with MVA shuttle plasmids containing MERS-CoV spike gene (explained above) to allow recombination with the MVA genome and deletion of dsRed marker whilst keeping the F11 promoter sequence. Recombinant MVA expressing MERS-CoV S protein was purified by plaque-picking and fluorescent selection using the sorting function of CyCLONE robotic module of a MoFlo Flow cytometer (Dako Cytomation, Denmark) as previously described (31). F11-MVAMERS and mH5-MVA MERS were confirmed to lack the native F11L ORF (and the dsRed marker), and contain MERS-CoV S by PCR (identity and purity PCR screening). The sequence of the S transgene amplified from these vaccines was confirmed. The recombinant viruses (vaccines) were amplified in 1500 cm² monolayers of CEFs cells, partially purified over sucrose cushions and titrated in CEFs cells according to standard practice, and purity and identity were again verified by PCR.

**Mouse immunogenicity**

Female BALB/c mice (Harlan, UK) aged 6 to 8 weeks were immunised intramuscularly (i.m.) in the upper leg (total volume 50 µL) with a total of 10^8 IU of ChAdOxi MERS with or without tPA or with a total of 10^6 pfu of either F11-MVAMERS or mH5-MVA MERS. For induction of short-term anaesthesia, animals were anaesthetised using vapourised IsoFloH. In prime only regimens, mice were vaccinated with ChAdOxi with blood samples taken at 14 days post immunisation (d.p.i) or 28 d.p.i. for serum isolation; and spleens were collected at 28 d.p.i. In heterologous prime-boost regimens, mice were vaccinated with ChAdOxi MERS and boosted with MVAMERS at 28 d.p.i; mice were bled at 28 d.p.i. (post-prime) or 42 d.p.i (14 days post-boost) for serum isolation, and spleens were collected at 42 d.p.i. In homologous regimens, mice were vaccinated with MVAMERS and boosted with MVAMERS at 21 d.p.i; mice were bled on 21 d.p.i. (post-prime) or 42 d.p.i (post-boost) for serum isolation and spleens were collected at 42 d.p.i.

**ELISpot, ICS, and flow cytometry**

Splenocytes were harvested for analysis by IFN-γ ELISpot or intracellular cytokine staining (ICS) and flow cytometry as previously described (35, 36), using re-stimulation with 2 µg/mL S291 MERS-CoV S-specific peptide (VYDTIKYYSIPHSI); for vaccine
cellular immunogenicity (37)); or 1 µg/mL E3 and F2(G) MVA vector-specific peptides (38) (for anti-MVA immune responses). In the absence of peptide re-stimulation, the frequency of IFN-γ+ cells, which was typically 0.1% by flow cytometry or less than 50 SFC by ELISpot, was subtracted from tested re-stimulated samples.

ELISA
2 µg/ml with capturing antigen (Si recombinant protein from MyBioSource, CA, USA) were used to coat ELISA plates, and standard endpoint ELISA protocol was followed, as previously described (39). Sera were prepared in a 10-fold serial dilution in PBS/T and then 50 µl were plated in duplicate wells. Serum from a naïve BALB/c mouse was included as a negative control. Goat anti-mouse total IgG conjugated to alkaline phosphatase (Sigma) and PNPP tablet (20 mg p-nitrophenylphosphate, SIGMA) substrate were used in the assay.

MERSpp Neutralisation assay
MERS pseudotyped viral particles (MERSpp) were produced and titrated using Huh7.5 cell line as described previously (40). For the MERSpp neutralization assay, serum samples were serially diluted in 96-well white plates (Nunc). A standard concentration of the MERSpp were added to the wells and plates were incubated for 1 h at 37 °C. After incubation, Huh7.5 cells (10,000 cells per well) were added to the plate in duplicates. Following 48 h incubation, cells were lysed and luciferase activity was measured. IC90 neutralisation titres were calculated for each mouse serum sample using GraphPad Prism.

Virus neutralisation assay
Induction of virus-neutralising antibodies was confirmed according to previously published protocols (37, 41). Briefly, mouse serum samples were tested for their capacity to neutralise MERS-CoV (EMC isolate) infections in vitro with 100 50% tissue culture infective doses (TCID₅₀) in Huh-7 cells. Sera of non-immunised mice served as negative control.

Statistical analysis
GraphPad Prism (GraphPad software) was used for statistical analysis and to plot data.

Ethics statement
All animal procedures were performed in accordance with the terms of the UKAnimals (Scientific Procedures) Act (ASPA) for the project licenses 30/2414 or 30/2889 and were approved by the University of Oxford Animal Care and Ethical Review Committee.

All mice were housed for at least 7 days for settlement prior to any procedure in the University animal facility, Oxford, UK under Specific Pathogen Free (SPF) conditions.

Results
Construction and antigen expression of MERS-CoV vaccine candidates
The spike gene from a camel isolate (Camel/Qatar_2_20i4 MERS-CoV isolate, GenBank accession number KJ650098.1) was cloned into four shuttle vectors that facilitate homologous recombination with the genome of ChAdOxi or MVA. Four recombinant viral vectors, two ChAdOxi and two MVA, were derived as described in the materials and methods. ChAdOxi based vaccine candidates were generated with or without the signal peptide of the human tissue plasminogen activator gene (tPA). The spike transgene expression in ChAdOxi MERS vaccine candidates is under the control of the human cytomegalovirus major immediate early promoter (CMV IE) that includes intron A. In MVAMERS vaccine candidates, the tPA was also inserted upstream of the spike transgene, which was under the control of either the ectopic MVA promotor or the endogenous F11 promoter (Figure 1A). All of our MERS-CoV vaccine candidates contain the same codon-optimized spike transgene. The expression of the newly synthesized transgene was first tested by transfection of an African green monkey kidney cell line (Vero cells) with the adenovirus shuttle vector, and immunofluorescence staining of the transfected cells (Figure 1B and 1C). This was performed to confirm the expression of the codon optimized spike transgene in mammalian cells. The level of transgene expression from the four vaccine candidates was not evaluated in vitro. We have previously reported that differences in MVA promoter activity detectable in vitro does not correlate with in vivo immunogenicity (31), and that only in vivo expression correlates with the in vivo immunogenicity.

Humoral Immunogenicity of ChAdOxi based MERS-CoV vaccine candidates

To evaluate humoral immune responses to ChAdOxi MERS with or without tPA, BALB/c mice were vaccinated with 1×10⁸ IU of ChAdOxi intramuscularly. Serum samples from 14 and 28 d.p.i. were collected and evaluated by ELISA. Both vaccine candidates induced a high level of Si-specific antibodies (mean endpoint titre (Log₁₀) = 4.8 with tPA, 4.7 without tPA), unlike the control vaccine, ChAdOxi encoding enhanced green fluorescent protein (ChAdOxi-eGFP, mean endpoint titre (Log₁₀) = 1). These antibody levels were similar between the two candidates (with or without tPA) at day 14. However, at 28 d.p.i. ChAdOxi MERS with tPA induced significantly higher Si-specific antibodies than ChAdOxi MERS without tPA (mean endpoint titre (Log₁₀) = 5.13 with tPA, 4.6 without tPA, Figure 2A). Serum samples from day 28 were selected for MERSpp neutralisation assay. Serum antibodies induced by ChAdOxi MERS with tPA showed significantly higher neutralisation activity than without tPA (mean titre IC₅₀ (Log₁₀) = 2.8 with tPA, 2.2 without tPA; Figure 2B). In order to confirm that the psuedotyped virus neutralisation assay was producing biologically relevant results, serum samples from mice immunised with ChAdOxi MERS with tPA were also tested.
in a neutralisation assay utilising wildtype MERS virus. This assay confirmed the neutralisation activity of mouse antibodies (nAb) with a median of 360 VNT (Virus Neutralization Test antibody titre; Figure 2C). We therefore continued to evaluate ChAdOxi MERS with tPA in addition to generating MVA MERS vaccine candidates with tPA.

**Cellular Immunogenicity of ChAdOxi based MERS-CoV vaccine candidates**

Having established the utility of tPA in ChAdOxi MERS vaccines (referred to as ChAdOxi MERS in the rest of this report) at increasing humoral responses, spleens were collected at 28 d.p.i. from immunised BALB/c mice. Splenocytes were processed to evaluate cellular immune responses to ChAdOxi MERS in ELISpot and Intracellular cytokine staining (ICS). Peptide S291, described by others (37), was used to re-stimulate the cells in both assays and ELISpot data showed a high level of IFN-γ secreting splenocytes (Median = 1300 SFU/10⁶ splenocytes; Figure 3A). ICS data confirmed the IFN-γ secreting CD8⁺ splenocytes also secreted TNF-α and IL-17 (Figure 3B).

**Immunogenicity of Heterologous ChAdOxi and MVA vaccination against MERS-CoV**

To evaluate humoral immune responses to heterologous prime-boost vaccination, BALB/c mice were immunised with ChAdOxi MERS vaccine and boosted with one of two different MVA MERS vaccine candidates four weeks later. The MVA based candidates differ in the promoters that controls the transgene expression: Fii-MVA MERS utilises the endogenous strong early F11 promoter and mH5-MVA MERS utilises the ectopic early/late mH5 promoter. Serum samples from 28 d.p.i. (post-prime) or 42 d.p.i. (post-boost) were collected and evaluated by ELISA and MERSpp neutralisation assay. At 28 d.p.i. ChAdOxi MERS induced similar levels of Si-specific antibodies and nAb as observed previously (Figure 4A and B). At 42 d.p.i. Si-specific antibodies were boosted to a higher level (mean endpoint titre (Log₁₀) = 5 by ChAdOxi MERS boosted to 5.8 by mH5-MVA MERS or 5.9 by F11-MVA MERS); Figure 4A) with nAb also enhanced to a statistically significant level (mean titre IC₉₀ (Log₁₀) = 2.87 by ChAdOxi MERS boosted to 3.3 by mH5-MVA MERS or 3.5 by F11-MVA MERS; Figure 4B). There was no difference in antibody levels induced using either the F11 or mH5 promoter in the MVA.

At 42 d.p.i. splenocytes were also processed to evaluate cellular immune responses to ChAdOxi MERS MVA MERS prime-boost vaccination in ELISpot and ICS as shown in Figure 3. The T cell responses to MERS S were boosted by the MVA vaccinations; in the ICS experiments, Fii-MVA and 1T1H5-MVA boosted the percentage of IFN-γ⁺ splenic CD8⁺ T cells to 7.3 and 5.2% respectively (Figure 4D) whereas the percentage was 2.5%
after ChAdOxi MERS prime in Figure 3B. The percentage of TNF-a+ splenic CD8+ T cells were also increased by MVAbost (comparing Figure 3B and 4D). Utilising the F11 promoter resulted in a trend towards greater cell-mediated immunogenicity (Figure 4C and D). Splenocytes were also re-stimulated with MVAbone-specific E3 and F(G)2 peptides and evaluated in ICS. Both MVAbased vaccines induced similar responses to E3 or to F(G)2 peptides, 2 weeks after MVAvaccination (Figure 4E and F). This similarity confirmed the efficiency of vaccine titration, vaccination, and sample processing because responses to each of those peptides are not expected to be different unless there is variation in the doses administered or sample preparation. Overall, MVAMERS vaccines were able to boost the humoral and cellular immune responses to ChAdOxi MERS prime vaccination. There was no difference between the F11 and \( \text{mH5} \) promoter in the resulting antibody titres after ChAdOxi prime/MVA boost, but there was a trend towards increased cellular immunogenicity when the F11 promoter was used.

**Immunogenicity of Homologous MVA vaccination against MERS-CoV**

To evaluate humoral immune responses to a homologous MVAMERS prime-boost vaccination, two groups of BALB/c mice were immunised with Fii-MVA MERS or mH5-MVA MERS and boosted with the same vaccine after three weeks. Serum samples from 21 d.p.i. (post-prime) or 42 d.p.i. (post-boost) were collected and evaluated in ELISA and MERSpp neutralisation assays. At 21 d.p.i. Fii-MVA MERS and \( \text{mH5-MVA} \) induced similar levels of Si-specific antibodies (mean endpoint titre \( \log_{10} \) = 3.2 and 2.8 respectively; Figure 5A). At 42 d.p.i Si-specific antibody levels had increased to 4.7 and 4.8 respectively (Figure 5A). The titres of nAb (MERS pp assay) were also similar for both vaccines (mean titre IC\(_{90} \) \( \log_{10} \) = 2.71 (Fii-MVA MERS) and 2.76 respectively; Figure 5B). Utilising different promoters in MVAvectors did not result in differences in the induced antibody levels. However, at 42 d.p.i. IFN-\( \gamma \) secreting splenocytes induced by Fii-MVA MERS were statistically significantly higher than those of mH5-MVA MERS ((Median = 525 and 249 SFU/10\(^6\) splenocytes, respectively, Figure 5C). Both MVAvaccines induced similar vector-specific immune responses as expected (Figure 5D and E).

**Summary**

Prior art vaccines against MERS-CoV have been developed and tested in a number of animal models (including non-human primates (42-44) and camels (45)) as well as in human clinical trials (46). All vaccine candidates focused on the spike antigen because it contains the receptor-binding domain used for cell entry by the virus, against which neutralising antibodies may be induced, and it is conserved. Therefore, our approach to the improvement of MERS-CoV vaccines focusses on platform and vaccination
regimens rather than antigen selection and optimisation. Here, we focused on using the same antigen (transgene) to develop a vaccine against MERS-CoV, and to assess different vectors, different versions of each vector, and different vaccination regimens. We generated a number of MERS-CoV vaccine candidates based on the same codon optimized spike transgene and ensured its expression in vitro before we evaluated the humoral and cellular immunogenicity in a pre-clinical BALB/c mouse model. ChAdOxi based vaccine candidates were produced with or without tPA. The tPA signal peptide was predicted to enhance the humoral immunogenicity of encoded vaccine antigens. Our data support this hypothesis and show a significant increase in the Si-specific antibody levels at 28 d.p.i. The level of neutralising antibodies was also increased when tPA was utilised. Thus it is clear that we have demonstrated a credible use as a human vaccine.

ChAdOxi MERS without tPA was still a potent vaccine candidate, inducing a high level of both Si-specific binding antibodies and MERS-CoV neutralising antibodies.

Neutralisation activity of mouse serum antibodies was assayed by using MERS-CoV pseudotyped viral particles (MERSpps), an approach used by a number of researchers for other human pathogens such as HIV, Influenza, and HCV to overcome the necessity of handling BSL-3 viruses (40). Additionally, we confirmed the ability of serum samples from vaccinated mice to neutralise live MERS virus. We therefore selected ChAdOxi MERS with tPA (simply referred to ChAdOxi MERS) for further evaluation.

ChAdOxi MERS also induced cellular responses for MERS S, with polyfunctional CD8+ T cells detected in the spleen of immunized mice. This supports the potency of the ChAdOxi viral vector in inducing T cellular immunity, observed previously in animal models (26, 32, 47) as well as in humans (33).

Following ChAdOxi prime/MVA boost, MVA significantly boosted the neutralizing antibody titres to higher levels. No difference in humoral immunity was found when either the F11 or nH5 promoter was used. Regarding the promoter effect on MVA cellular immunogenicity, we have previously reported that utilising the F11 promoter enhanced malaria and influenza antigens in MVA (31). Here, we again report that F11-MVAMERS induced higher T cell responses than nH5-MVA MERS in a homologous prime-boost MVAMERS vaccination.

All of our vaccine candidates induced humoral (with nAb) and cellular immune (with polyfunctional CD8+ T cell) responses against MERS-CoV spike antigen. Effects on immunogenicity of different versions of the vaccines were noted, with the use of the tPA leader sequence in ChAdOxi, and the use of the F11 promoter in MVA producing increases in immunogenicity compared to no leader sequence, or the nH5 promoter.
The protective level of either antibodies or cellular immunity required to counter MERS-CoV infection in humans or in animal models is not yet defined, despite some efforts (48-51). The ideal vaccine would provide rapid onset of immunity and complete protective efficacy after a single dose, with a long duration of immunity. Complete protective efficacy of one dose of ChAdOxi expressing the external glycoprotein of Rift Valley Fever Virus has been demonstrated in multiple species and it is already known that ChAdOxi RVF is highly immunogenic in camels (32). However, RVF is a completely different viral family to the coronaviruses (as discussed above).

To date, the only vaccine against MERS to be tested in camels is an MVA vectored vaccine (41) which was protective in hDPP4 transgenic mice immunized with a homologous prime/boost regimen (37) but in camels required two doses given both intranasally and intramuscularly to provide partial protection and reduction of virus shedding (45). Here we show that a single dose of ChAdOxi MERS is as immunogenic as two doses of MVAMERS, demonstrating that this regimen should be tested for protective efficacy in camels. However if this is not completely protective, administration of MVAMERS as a heterologous boost may be employed.

In our hands one dose of MVA resulted in an endpoint titre of 3 logs, two doses of MVA produced 4.7 logs, one dose of ChAdOxi produced 5 logs, and ChAdOxi/MVA prime boost produced 5.9 logs, showing that the invention delivers technical benefits.

If a two dose regimen is required, e.g. for particular applications such as sustained immunity in healthcare workers, ChAdOxi/MVA would be more likely to provide complete protection than MVA/MVA.

ChAdOxi MERS is thus demonstrated to be a plausible and credible vaccine for both camels and humans.

References


EXAMPLE 2: Protective Efficacy of a Novel Simian Adenovirus Vaccine Against Lethal MERS-CoV Challenge in a Transgenic Human DPP4 Mouse Model: A Simian Adenovirus MERS-CoV Vaccine

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel zoonotic virus that causes severe respiratory disease in humans with case fatality rates close to 40%, but for which no vaccines are available. Here, we evaluated the utility of ChAdOxi, a promising replication-deficient simian adenovirus vaccine vector platform with an established safety profile in humans and dromedary camels, for MERS-CoV vaccine development. Using a transgenic lethal BALB/c MERS-CoV mouse model we show that single dose intranasal or intramuscular immunization with ChAdOxi MERS, encoding
full-length MERS-CoV Spike glycoprotein, is highly immunogenic and confers protection against lethal viral challenge. Immunogenicity and efficacy were comparable between immunisation routes. Together these data show credible utility of ChAdOxi MERS vaccine in humans and dromedary camels, the animal reservoir of infection.

One ChAd vector, termed ChAdOxi δ, has undergone testing in dromedary camels, showing excellent safety and immunogenicity when encoding Rift Valley Fever viral glycoproteins δ. Here, using standard adenovirus production methods δ we made a vaccine construct, ChAdOxi MERS, encoding the full-length MERS-CoV spike glycoprotein (GenBank accession number KJ650098.1) targeted by protective neutralising antibodies δ. To determine vaccine immunogenicity and efficacy we utilised a recently developed transgenic lethal BALB/c mouse model expressing the human dipeptidyl peptidase (hDPP4) gene in the Rosa26 locus, which renders mice susceptible to MERS-CoV infection δδ.

hDPP4 mice were vaccinated intranasally or intramuscularly with $10^8$ infectious units of either a control ChAdOxi vaccine encoding enhanced green fluorescent protein (ChAdOxi GFP) or the ChAdOxi MERS vaccine. Sera were obtained before vaccination and 28 days post-vaccination. ChAdOxi MERS vaccination elicited high titre neutralizing antibodies with both immunisation routes being equally immunogenic (Kolomgorov-Smirnov test, $p=0.657i$) (Fig. 6A). No MERS-CoV neutralizing antibody response was observed among the ChAdOxi GFP vaccinees.

To evaluate vaccine efficacy animals were challenged intranasally with $10^4$ TCID$_{50}$ of the HCoV-EMC/2012 MERS-CoV strain in a total volume of 25 µl and observed daily for signs of disease. Euthanasia was indicated at >20% loss of initial body weight. At 3 days post-inoculation (dpi), four animals from each group were sacrificed and lungs collected for analyses. The remaining six animals per group were sacrificed 28 dpi, or when they reached the humane endpoint criteria. ChAdOxi GFP vaccinees developed signs of disease, including loss of body weight, ruffled fur and lethargy (Fig. 6B). Weight loss begun 3 dpi and at 7-8 dpi all mice in the ChAdOxi GFP groups either succumbed to infection or reached the predefined euthanasia endpoint (Figure 7C). No signs of disease or significant loss of body weight were observed in mice vaccinated with ChAdOxi MERS (Fig. 6B and C).

The presence of MERS-CoV RNA in the lungs was analyzed by qRT-PCR on mice (n=4/group) sacrificed at 3 dpi. High viral loads were found in the lower respiratory tract of the ChAdOxi GFP vaccinated mice (intranasal $10^{5.32}$ TCID$_{50}$/gram tissue, 95% confidence interval (CI): $10^{2.28}$ to $10^{5.77}$, intramuscular $10^{4.51}$ TCID$_{50}$/gram tissue, 95% CI: $10^4$ to $10^4.96$). No viral RNA was detected in any of the ChAdOxi MERS vaccinated...
mice (Figure 6D). Immunohistochemistry staining for MERS-CoV in lung tissue showed abundance of antigen in the ChAdOxi GFP vaccinated mice, but not the ChAdOxi-MERS vaccinees (Figure 7). MERS-CoV staining was observed in the type I and type II pneumocytes (Figure 7 inserts) but not in any of the other respiratory cells such as endothelial cells, bronchiolar epithelium or macrophages. Together these data provide support for applicability of ChAdOxi MERS vaccine in humans and dromedary camels. This is advantageous given the established safety profile of the ChAdOxi platform in humans and dromedary camels. A deployable human MERS-CoV vaccine will need to be safe and efficacious in at-risk populations, including healthcare workers, camel herders and those with comorbidities as highlighted in the ongoing WHO-led consultation on an ideal target product profile for MERS-CoV vaccines.

ChAdOxi MERS induces T cell responses against MERS Spike protein as well as neutralizing antibodies (example 1). Identification of immune correlates of protection against MERS-CoV in humans and camels allows cost-effective disease surveillance and vaccine monitoring.

In summary, we have demonstrated the utility of the ChAdOxi platform for MERS-CoV vaccine development in a lethal mouse model. The excellent immunogenicity and efficacy observed here show the value of ChAdOxi MERS in dromedary camels and humans.

Ethics statement
 Approval of animal experiments was obtained from the Institutional Animal Care and Use Committee of the Rocky Mountain Laboratories. The performance of experiments was done following the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) by certified staff in an AAALAC-approved facility, following the guidelines and basic principles in the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals. Work with infectious MERS-CoV strains under BSL3 conditions was approved by the Institutional Biosafety Committee (IBC). Inactivation and removal of samples from high containment was performed according to IBC-approved standards.

REFERENCES TO EXAMPLE 2
1. SYNOPSIS

**Trial Title**  
A phase I clinical trial to determine the safety and immunogenicity of the candidate MERS-CoV vaccine ChAdOxi MERS-S in UK healthy adult volunteers.

**Trial Centre**  
Centre for Clinical Vaccinology & Tropical Medicine, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE

**Trial Identifier**  
MERS 001

**Clinical phase**  
I

**Study Design**  
Open-labelled, non-randomised, dose escalation, phase I clinical trial

**Population**  
Healthy adults aged 18 - 50 years

**Planned Sample Size**  
24 volunteers

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of ChAdOxi MERS-S</th>
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<tbody>
<tr>
<td>Group 1 (n=6)</td>
<td>5 x 10⁹ vp</td>
</tr>
<tr>
<td>Group 2 (n=9)</td>
<td>2.5 x 10⁹ vp</td>
</tr>
<tr>
<td>Group 3 (n=9)</td>
<td>5 x 10⁹ vp</td>
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</tbody>
</table>

**Follow-up duration**  
26 weeks post vaccine administration

**Planned Trial Period**  
Q4 2017 to Q4 2018

**Primary Objective**  
To assess the safety profile of the candidate vaccine ChAdOxi MERS-S in healthy adult volunteers

**Secondary Objective**  
To assess the immunogenicity of the candidate vaccine ChAdOxi
MERS-S in healthy adult volunteers

**Investigational Products**
ChAdOxi MERS-S, a replication-deficient simian adenoviral vector expressing the spike (S) protein of MERS Coronavirus.

**Dose per Administration**
- ChAdOxi MERS-S 5 x 10^9 vp
- ChAdOxi MERS-S 2.5 x 10^10 vp
- ChAdOxi MERS-S 5 x 10^10 vp

**Form**
Liquid (all finished products)

**Route**
Intramuscularly (IM) into the deltoid region of the arm

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2. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>AR</td>
<td>Adverse reaction</td>
</tr>
<tr>
<td>CBF</td>
<td>Clinical Bio-Manufacturing Facility</td>
</tr>
<tr>
<td>CCVTM</td>
<td>Centre for Clinical Vaccinology and Tropical Medicine</td>
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<tr>
<td>ChAdOxi</td>
<td>Chimpanzee Adenovirus Oxi</td>
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<tr>
<td>ChAdOxi MERS-S antigen</td>
<td>Recombinant Chimpanzee Adenovirus Oxi with MERS spike antigen</td>
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<tr>
<td>CI</td>
<td>Chief Investigator</td>
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<tr>
<td>CRF</td>
<td>Case report form or Clinical Research Facility</td>
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<tr>
<td>CTRG</td>
<td>Clinical Trials Research Governance</td>
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<td>Da</td>
<td>Dalton</td>
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<td>DSUR</td>
<td>Development Safety Update Report</td>
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<td>EC</td>
<td>Ethics committee</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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<td>ELISPOT</td>
<td>Enzyme linked immunospot assay</td>
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<tr>
<td>FBC</td>
<td>Full blood count</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
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<tr>
<td>GMO</td>
<td>Genetically modified organism</td>
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<tr>
<td>GMP</td>
<td>Good Manufacturing Practice</td>
</tr>
<tr>
<td>HA</td>
<td>Haemagglutinin</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B surface antigen</td>
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3. BACKGROUND & RATIONALE

3.1 Impact of MERS-CoV and the need for a vaccine
The dipeptidyl peptidase 4 (DPP4) receptor is used by the MERS-CoV virus during infection and is highly conserved between Camels and Humans. The MERS-CoV spike (S) protein is a characteristic structural component of the virion membrane and its Si domain mediates binding to DDP4. The spike protein has been chosen as the target antigen for use in the replication-deficient simian adenovirus developed by the University of Oxford, ChAdOxi vaccine vector. ChAdOxi has shown successful results in the development of Oxford lead vaccines which have gone onto enter phase I trials within the UK (7). In this study we propose the manufacture to GMP of the ChAdOxi MERS-S vaccine and its use in a small open labelled phase I trial in Oxford.

3.1. Progress towards a MERS vaccine

Global efforts to develop a coronavirus vaccine faded in the aftermath of SARS-CoV pandemic but has since gained renewed momentum in the face of the current MERS-CoV outbreak. Most of the developed vaccines were based on the S surface glycoprotein, the primary target for neutralizing antibodies during any natural coronavirus infection. A number of preclinical and clinical studies showed that the SARS-CoV Si protein subunit, and specifically the RBD at its core, could serve as a dominant target for neutralizing antibodies in mice, non-human primates, and humans. Si, therefore, became the basis for a number of promising SARS-CoV vaccine candidates (8).

The Si protein subunit and RBD have also been the basis for several MERS-CoV vaccine candidates. Both constructs have elicited neutralizing antibodies of high potency across multiple viral strains. Despite their demonstrated immunogenicity in animal models and anticipated safety in humans, RBD or Si-subunit based vaccine candidates are limited in their epitope breadth. Vaccine candidates that elicit a more diverse antibody repertoire as well as a robust cellular immuneresponse may offer the advantage of broader and more durable protection (8).

Live attenuated viruses have historically been among the most immunogenic platforms available, as they have the capacity to present multiple antigens across the viral life cycle in their native conformations. However, manufacturing live-attenuated viruses requires complex containment and biosafety measures. Furthermore, live-attenuated viruses carry the risks of inadequate attenuation causing disseminated disease, particularly in immunocompromised hosts. Given that moderately immuno-compromised adults with co-morbidities have suffered the most severe MERS-CoV disease, making a live-attenuated virus vaccine is a less viable option. Replication competent viral vectors could pose a similar threat for disseminated disease in the immuno-suppressed. Replication deficient vectors, however, avoid that risk while
maintaining the advantages of native antigen presentation, elicitation of T cell immunity and the ability to express multiple antigens (8). Several recombinant DNA, protein and viral vectored MERS candidate vaccines have been developed and tested in animal models (mice, non-human primates and camels) with varied efficacy results. Recently, a recombinant MVA encoding the full length Spike protein antigen (S) showed partial efficacy by significantly reducing MERS-CoV viral shedding in a camel challenge study. (9, 10) The first MERS-CoV vaccine to be used in humans has recently entered a phase I dose ranging safety study in January 2016. The GLS-5300, a DNA plasmid vaccine that expresses the MERS-CoV spike (S) glycoprotein, is being administered to 75 healthy adult volunteers in the USA, by the Walter Reed Army Institute of Research. Safety and immunogenicity data are expected to be reported by the end of 2017.

3.2. MERS spike protein as a vaccine antigen

Coronaviruses (CoVs) are spherical and enveloped viruses with large, unsegmented, single positive RNA genomes. One-third of the genome is responsible for coding the structural proteins: spike (S) glycoprotein, small envelope protein (E), integral membrane protein (M), and genome-associated nucleocapsid protein (N). The proteins E, M, and N are mainly responsible for the assembly of the virions, while the S protein is involved in receptor binding and bears membrane fusion capabilities during CoVs infection. Thus, the S protein has an essential role in virus entry and determines tissue and cell tropism, as well as host range (11). S is a type I, trimeric, transmembrane protein located at the surface of the viral envelope, giving rise to spikeshaped protrusions from the virion. S is 1353 amino acids in length, heavily glycosylated (with 21 predicted N-linked glycosylation sites), and consists of a large ectodomain and a short cytosolic tail. The S proteins of CoVs can be divided into two functional subunits: the N-terminal S1 subunit forms the globular head, and the membrane-embedded C-terminal S2 (11). S1 and S2 subunits are respectively, responsible for cellular receptor DPP4 binding via the receptor-binding domain (RBD), and fusion of virus and cell membranes, thereby mediating the entry of MERS-CoV into the target cells. The MERS-CoV RBD consists of a core structure, which is homologous to that of the SARS-CoV S protein RBD, and a receptor-binding motif, which is unique to MERS-CoV, thus determining viral pathogenesis and receptor recognition (12). The roles of S in receptor binding and membrane fusion make it a perfect target for vaccine and antiviral development. Previous studies on SARS-CoV reveal that vaccines based on the S protein can induce antibodies to block virus binding and fusion or
neutralize virus infection (11). ChAdOxi MERS-S expresses a codon-optimised coding sequence for Spike protein from the MERS-CoV isolate Camel/Qatar_2_2014 (GenBank:KJ650098.i).

3.3 Adenovirus-vectored Vaccines

Adenoviruses are attractive vectors for human vaccination. They possess a stable genome so that inserts of foreign genes are not deleted and they can infect large numbers of cells without any evidence of insertional mutagenesis.

Replication defective adenovirus can be engineered by deletion of genes from the El locus, which is required for viral replication, and these viruses can be propagated easily with good yields in cell lines expressing El from AdHu5 such as human embryonic kidney cells 293 (HEK 293 cells) (13).

Previous mass vaccination campaigns in over 2 million adult US military personnel using orally administered live human adenovirus serotype 4 and 7 have shown good safety and efficacy data (14). Human adenoviruses are under development as vectors for malaria, HIV and hepatitis C vaccines, amongst others. They have been used extensively in human trials with excellent safety profile mainly as vectors for HIV vaccines.

A limiting factor to widespread use of human adenovirus as vaccine vectors has been the level of anti-vector immunity present in humans where adenovirus is a ubiquitous infection. The prevalence of immunity to human adenoviruses prompted the consideration of simian adenoviruses as vectors, as they exhibit hexon structures homologous to human adenoviruses (15). Simian adenoviruses are not known to cause pathological illness in humans and the prevalence of antibodies to chimpanzee origin adenoviruses is less than 5% in humans residing in the US.

3.4 ChAdOxi

ChAdOxi is a novel recombinant chimpanzee adenovirus designed as a vaccine vector, developed by The Jenner Institute at the University of Oxford. This viral vector has been used by researchers at the University of Oxford to produce a number of vaccines expressing a range of different antigens. Three phase I clinical trials have been completed in the UK using ChAdOxi with different inserts (two influenza trials and one TB trial).

ChAdOxi is produced from a replication-deficient (El and E3 deleted) simian adenovirus and it has been described by Dicks et al (16). The vector was constructed in a bacterial artificial chromosome (BAC) to facilitate genetic manipulation of genomic clones with improved stability and flexibility. Cellular immunogenicity of recombinant Ei E3-deleted ChAdOxi was comparable to that of other species E derived chimpanzee
adenovirus vectors including ChAd63, the first simian adenovirus vector to enter clinical trials in humans. The Ei region is essential for viral replication, hence the ability to delete Ei renders the new vector immediately replication incompetent. The deletion of the non-essential adenovirus E3 region increases the insert capacity of the new vector by approximately 5Mb. It is known that the proteins encoded by the E4 region of adenoviruses interact with Ei during viral replication, and the imperfect interaction between the gene products of the AdHu5 Ei gene produced by HEK293 cells and simian E4 gene products has been found to result in impaired viral replication in this cell line, and consequently lower virus yields. In ChAdOxi, Ad5 E40rf4 has been inserted to replace the homologous simian virus coding sequence, resulting in improved viral replication during vaccine production. Since no replication of the virus takes place after immunization, this replacement has no effect on immunogenicity of the viral vector. Insertion of recombinant antigens at the Ei locus is performed using Gateway® site specific recombination technology (Invitrogen).

3.5 Development of ChAdOxi MERS-S
ChAdOxi MERS-S encodes the Spike (S) surface glycoprotein of the coronavirus. A genomic clone of ChAdOxi MERS-S was prepared by Gateway® recombination between an entry plasmid containing the codon-optimised coding sequence for Spike protein from the MERS-CoV isolate Camel/Qatar_2_20i4 (GenBank:KJ650098.i), and the Ei-and E3-deleted ChAdOxi destination vector.

3.6 Preclinical Studies
3.6.1 Efficacy and Immunogenicity
Mice (balb/c) were immunised with ChAdOxi or MVA vectored vaccines expressing MERS-CoV Spike protein. Serum samples were taken after 28 days and endpoint titres measured by ELISA. This study showed that a single dose of ChAdOxi results in equivalent immunogenicity to two doses of MVA.

Fig. 8 shows Immunogenicity of viral vectored vaccines MERS vaccines in mice.

An efficacy preclinical study has been conducted where mice transgenic for the hDPP4 receptor were immunised with a single dose of ChAdOxi MERS-S by either intranasal or intramuscular injection. The control ChAdOxi vaccine expressed eGFP as the vaccine antigen. Serum neutralising titres were measured 28 days after vaccination, when the mice were then challenged with MERS CoV by intranasal inoculation. The results showed that mice immunised with the MERS vaccine by either route were
completely protected against MERS CoV infection. No virus was detected in the lungs of the mice receiving the MERS vaccine and they all survived, whereas all of the sham-vaccinated mice succumbed to infection within 8 days (Vincent Munster, unpublished data).

Fig. 9 A. Virus neutralising titres in mice amongst ChAdOxi MERS-S and controls administered via intranasal or intramuscularly. B. Viral load after MERS-CoV challenge. C Survival amongst ChAdOxi MERS-S and control mice after intranasal MERS-CoV challenge.

3.6.2 Toxicity
A GLP toxicology study performed on BALB/c mice revealed that intramuscular administration of ChAdOxi MERS-S is safe and well tolerated. Clinical observations, inoculation site reactogenicity, clinical chemistry, clinical haematology, gross necropsy, organ weights and histopathology indicated no overt toxicity related to ChAdOxi MERS-S vaccine administration.

3.7 Previous clinical experience
This will be the first-in-human study employing ChAdOxi MERS-S. However, ChAdOxi vectored vaccines expressing different inserts have previously been used in 126 healthy volunteers taking part in clinical trials conducted by the University of Oxford in the UK (table 1). ChAdOxi encoding the influenza fusion protein NP+Mi has been safely administered to 84 healthy adult volunteers in the UK in two completed clinical trials conducted at The Jenner Institute (FLU004 and FLU005). FLU004 was a phase I, open-label, non-randomised dose escalation study of ChAdOxi NP+Mi. The vaccine was safe, well tolerated and immunogenic, inducing ELISpot responses at all doses. The dose of $2.5 \times 10^{10}$ vp was chosen for further studies of ChAdOxi NP+Mi (7). FLU005 was a multicentre phase I, randomised study to determine the safety and immunogenicity of vaccination regimens employing the candidate influenza vaccines MVA-NP+Mi and ChAdOxi NP+Mi. Sixty-nine (69) healthy adult volunteers have received ChAdOxi NP+Mi at a concentration of $2.5 \times 10^{10}$ vp. Administrations of ChAdOxi NP+Mi and MVA-NP+Mi vaccines were found to be safe and well-tolerated, in agreement with our previous studies (7, 17-20). The majority of adverse events were mild to moderate in nature and lasted for 1-2 days. The most common local adverse
event was arm pain at the site of injection and the most common systemic adverse event was mild fatigue and headache.

TB034 was an open-label, phase I clinical trial in which 42 healthy adult volunteers received the ChAdOxi viral vector expressing the \textit{Mycobacterium tuberculosis} antigen 85A (ChAdOxi 85A). No major safety concerns associated with ChAdOxi 85A administration have been reported.

ChAdOxi 5T4 has been given in the VANCEoi study which is an ongoing first-in-man open label randomized phase I study to determine the safety and immunogenicity of heterologous prime boost ChAd-MVA vaccination against oncofetal antigen 5T4. To date, 34 participants have received the ChAdOxi 5T4 vaccine at a dose of $2.5 \times 10^{10}$ vp and only mild AEs related to the vaccination have been reported. VAC067 is an ongoing first-in-man study of the ChAdOxi viral vector expressing dual second generation liver-stage malaria antigens LSAi and LSAP2 (ChAdOxi LS2). No significant safety concerns have been reported until this date.

None of the above mentioned clinical trials reported serious adverse events associated with the administration of ChAdOxi, which was shown to have a good safety profile.

\textbf{Table 1.} Clinical experience with ChAdOxi viral vector vaccines.

<table>
<thead>
<tr>
<th>Country</th>
<th>Trial</th>
<th>Vaccine</th>
<th>Age</th>
<th>Route</th>
<th>Dose</th>
<th>Number of Volunteers (Received ChAdOxi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>FLU004</td>
<td>ChAdOxi NP+Mi</td>
<td>18-50</td>
<td>IM</td>
<td>$5 \times 10^8$ vp</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$5 \times 10^9$ vp</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$2.5 \times 10^{10}$ vp</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$5 \times 10^{10}$ vp</td>
<td>6</td>
</tr>
<tr>
<td>UK</td>
<td>FLU005</td>
<td>ChAdOxi NP+Mi MVANP+Mi</td>
<td>18-50</td>
<td>IM</td>
<td>$2.5 \times 10^{10}$ vp</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(week 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ChAdOxi NP+Mi MVANP+Mi</td>
<td>18-50</td>
<td>IM</td>
<td>$2.5 \times 10^{10}$ vp</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(week 52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVANP+Mi ChAdOxi NP+Mi</td>
<td>18-50</td>
<td>IM</td>
<td>$2.5 \times 10^{10}$ vp</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(week 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVANP+Mi ChAdOxi NP+Mi</td>
<td>18-50</td>
<td>IM</td>
<td>$2.5 \times 10^{10}$ vp</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(week 52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ChAdOxi NP+Mi >50 IM 2.5x10^10 vp 12
ChAdOxi NP+Mi MVANP+Mi (week 8) >50 IM 2.5x10^10 vp 12
MVANP+Mi (week 8) ChAdOxi 85A to support a further phase I and phase II study with our collaborators based in the Middle East. This study would be

3.8 Rationale

MERS-CoV is an emerging zoonotic viral disease considered a global threat and listed as a priority pathogen for urgent Research and Development. The recent MERS-CoV outbreaks in the Middle East (from 2012 and still ongoing) and South Korea (2015) have caused a total of 652 deaths representing a case fatality rate of approximately 36% and imported cases have now been reported in 27 countries.

Chimpanzee adenovirus vaccine vectors have been safely administered to over 1000 people using a wide range of infectious disease targets including malaria (21), HIV (22), tuberculosis, influenza (7), hepatitis C (23), RSV (24) and, most recently, Ebola (25). ChAdOxi viral vectored vaccines have shown to be both safe and immunogenic in previous clinical trials in the UK (FLU004, FLU005 and TB034). Single-dose immunisation with ChAdOxi MERS-S vaccine has shown to elicit high levels of neutralising antibody in animal model.

Finally, the One Health vaccine development approach used here, by which the same vaccine is co-developed for humans and susceptible animal species, is well suited to many emerging outbreak pathogens, most of which involve zoonotic transmission (26). The approach allows the possibility of cost reductions for the final product by increasing the scale of manufacture (27). Ultimately the vaccine could be licensed for use in camels in the Middle East and North Africa. If licensed, human vaccines could be deployed for occupationally exposed individuals such as camel workers and health care professionals, with stockpiles available for use in the case of an outbreak.

3.9 Vaccine Development Strategy

The data from this first in human study will then be used to support a further phase I and phase II study with our collaborators based in the Middle East. This study would be
ran in parallel with a study in camels with our collaborators at Qassim University, Saudi Arabia. Testing of the MERS vaccine in camels is funded by the Liverpool School of Tropical Medicine Confidence in Concept Scheme.

4. STUDY OVERVIEW

This is a first-in-human, open-label, dose escalation, phase I clinical trial to assess the safety and immunogenicity of the candidate ChAdOxi MERS-S vaccine in healthy volunteers aged 18-50. The vaccine will be administered intramuscularly.

Volunteers will be recruited and vaccinated at the CCVTM, Oxford. There will be 3 study groups and a total of 24 volunteers will be enrolled (table 2). Staggered enrolment will apply for the first three volunteers within each group. Volunteers will be first recruited into Group 1 and subsequently into Groups 2 and 3 following interim clinical safety reviews (see section 8.4.2). Volunteers will be allocated to a study group by selecting eligible volunteers for enrolment in the order in which they were deemed eligible, following screening.

4.1 Rationale for Selected Doses

Doses to be administered in this trial have been selected on the basis of clinical experience with the ChAdOxi adenovirus vector expressing different inserts and similar adenovirus vectored vaccines (eg. ChAd63).

A first-in-man dose escalation study using the ChAdOxi vector encoding an influenza antigen (FLU004), safely administered ChAdOxi NP+Mi at doses ranging from $5 \times 10^8$ to $5 \times 10^{10}$ vp. Subsequent review of the data identified an optimal dose of $2.5 \times 10^{10}$ vp balancing immunogenicity and reactogenicity. This dose has subsequently been given to over 100 volunteers in numerous larger phase 1 studies at the Jenner Institute (FLU005, TB034 and VANCE01) and ChAdOxi vectored vaccines have thus far demonstrated to be very well tolerated. The vast majority of AEs have been mild-moderate and there have been no SARs until this date.

Another simian adenovirus vector (ChAd63) has been safely administered at doses up to $2 \times 10^{11}$ vp with an optimal dose of $5 \times 10^{10}$ vp, balancing immunogenicity and reactogenicity.

As this is a first-in-human assessment of the MERS-S antigenic insert, the first dose of ChAdOxi MERS-S proposed in this study ($5 \times 10^9$ vp) is therefore at least 10 fold less than what this new insert is expected to be tolerated ($5 \times 10^{10}$ vp). Doses will be
gradually increased aiming to provide an optimal dose of ChAdOxi MERS-S considering the tolerability, reactogenicity and immunogenicity profiles.

4.2 Study Groups

Table 2. Study Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Single Dose ChAdOxi MERS-S</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=6)</td>
<td>$5 \times 10^9$ vp</td>
<td>IM</td>
</tr>
<tr>
<td>Group 2 (n=9)</td>
<td>$2.5 \times 10^{10}$ vp</td>
<td>IM</td>
</tr>
<tr>
<td>Group 3 (n=9)</td>
<td>$5 \times 10^{10}$ vp</td>
<td>IM</td>
</tr>
</tbody>
</table>

4.2.1 First Volunteers

Volunteers will be enrolled and doses will be escalated according to the plan outlined below.

The first volunteer in the study will receive $5 \times 10^9$ vp of ChAdOxi MERS-S (group 1). This volunteer will be vaccinated ahead of any other volunteers and the profile of adverse events will be examined after 48h. Provided there are no safety concerns as assessed by the Chief Investigator (CI) and the Local Safety Monitor (LSM), another 2 volunteers will be vaccinated at the same dose after at least 48 hours has elapsed following vaccination of the first volunteer and at least 1 hour apart from each other. An independent safety review will be conducted by the LSM after vaccination of the first 3 volunteers in group 1. This review will include the results of safety blood tests at day 7 post vaccination and an assessment of the profile of the adverse events reported. The CI and the LSM will be asked to provide the decision on whether to proceed with vaccinations of the remaining participants in group 1 and the first volunteer to receive the next incremental dose in group 2. If there are no safety concerns, the remaining volunteers in Group 1 and the first volunteer in group 2 may be vaccinated.

The same procedure will apply for each of the first 3 volunteers enrolled at higher dosage groups and prior to dose escalation (groups 2 and 3).

4.2.2 Duration of study

The total duration of the study will be 26 weeks from the day of enrolment for all volunteers.

4.2.3 Definition of Start and End of Trial
The start of the trial is defined as the date of the first vaccination of the first volunteer. The end of the trial is the date of the last visit of the last volunteer.

4.3 Potential Risks for volunteers
The potential risk to participants is considered as low. The potential risks are those associated with phlebotomy and vaccination. In general, recombinant adenoviral vectors are safe. Similar vaccines encoding different antigens have been given to several thousand volunteers (including children) with a good safety profile.

Phlebotomy:
The maximum volume of blood drawn over the study period (approximately 500ml) should not compromise these otherwise healthy volunteers. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venepuncture, which will not be documented as AEs if they occur.

Vaccination:
ChAdOxi MERS-S has not been used in humans before and therefore will be initially administered at the lower dose of $5 \times 10^9$ vp before progressing to the higher doses of $2.5 \times 10^9$ and $5 \times 10^9$ in Groups 2 and 3. Potential expected risks from vaccination include local effects such as pain, redness, warmth, swelling, tenderness or itching.

Systemic reactions that could potentially occur following immunisation with a recombinant adenovirus vaccine include a flu-like illness with feverishness, fatigue, malaise, arthralgia, myalgia and headache.

As with any vaccine, Guillain-Barre syndrome or immune-mediated reactions that can lead to organ damage may occur, but this should be extremely rare. Serious allergic reactions including anaphylaxis could also occur and for this reason volunteers will be vaccinated in a clinical area where Advanced Life Support trained physicians, equipment and drugs are immediately available for the management of any serious adverse reactions (SAR).

4.4 Known Potential Benefits
Volunteers will not benefit directly from participation in this study. However, it is hoped that the information gained from this study will contribute to the development of a safe and effective MERS vaccine regime. The only benefits for participants would be information about their general health status.

5 OBJECTIVES AND ENDPOINTS
The number of volunteers has been chosen to generate adequate safety and immunogenicity data to meet these objectives, whilst minimising the number of volunteers exposed to a new vaccination regimen.

5.1 Primary Objective
To assess the safety and tolerability of ChAdOxi MERS-S in healthy volunteers.

5.1.1 Primary Outcome Measures
The specific endpoints for safety and reactogenicity will be actively and passively collected data on adverse events.

The following parameters will be assessed for all study groups:
- Occurrence of solicited local reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of unsolicited adverse events for 28 days following the vaccination
- Change from baseline for safety laboratory measures
- Occurrence of serious adverse events during the whole study duration

Volunteers will undergo clinical follow up for adverse events for a further 182 days following completion of the vaccination regimen. SAEs will be collected throughout the study. The duration of follow up reflects the desire to obtain longer term safety data with the first use of ChAdOxi MERS-S in humans.

5.2 Secondary Objective
To assess the cellular and humoral immunogenicity of ChAdOxi MERS-S in healthy adult volunteers.

5.2.1 Secondary Outcome Measures
Measures of immunogenicity to the ChAdOxi MERS-S vaccine may include:
- ELISA to quantify antibodies to MERS-S protein
- Ex vivo ELISpot responses to MERS-S

Other exploratory immunology may be carried out in collaboration with other specialist laboratories, including laboratories outside of Europe. This would involve transfer of serum/plasma and/or peripheral blood mononuclear cells (PBMC), but samples would be anonymised. Volunteers will be consented for this.

6 INVESTIGATIONAL PRODUCTS
The following vaccinations will be given in this study:

1. ChAdOxi MERS-S 5 x 10^8vp
2. ChAdOxi MERS-S 2.5 x 10^9vp
3. ChAdOxi MERS-S 5 x 10^9vp
4. ChAdOxi MERS-S 2.5 x 10^9vp
5. ChAdOxi MERS-S 5 x 10^9vp

6.1. Manufacturing and Presentation

6.1.1 Description of ChAdOxi MERS-S

ChAdOxi MERS-S vaccine consists of the replication-deficient simian adenovirus vector ChAdOxi, containing the structural surface glycoprotein (Spike protein) antigens of the MERS-CoV expressed from the strong CMV IE promoter.

6.1.2. ChAdOxi MERS-S formulation and packaging

ChAdOxi MERS-S is manufactured in formulation buffer at a nominal concentration of >1 x 10^11 vp/mL. The drug product is filled into 2mL glass vials with a 13 mm grey bromobutyl rubber freeze-dry stopper (CE Marked, supplied by Adelphi Tubes) and a 13 mm aluminium seal. The nitrogen filled vials are supplied sterile. The containers and closures are tested for compliance with defined specifications. The vials are made from Ph Eur Type 1 glass.

6.2 Supply

ChAdOxi MERS-S has been formulated and vailed under Good Manufacturing Practice conditions at the Clinical Bio-Manufacturing Facility (CBF), University of Oxford. At the CBF the vaccine will be certified and labelled for the trial by a Qualified Person (QP) before transfer to the clinical site.

6.3 Storage

The vaccine is stored at nominal -80°C in a locked freezer, at the clinical site. All movements of the study vaccines will be documented in accordance with existing standard operating procedure (SOP). Vaccine accountability, storage, shipment and handling will be in accordance with relevant SOPs and forms.

6.4 Administration of Investigational Medicinal Products

On vaccination day, ChAdOxi MERS-S will be allowed to thaw to room temperature and will be administered within 1 hour of removal from the freezer. The vaccine will be administered intramuscularly into the deltoid of the non-dominant arm (preferably). All volunteers will be observed in the unit for 1 hour (±10 minutes) after vaccination. During administration of the investigational products, Advanced Life Support drugs
and resuscitation equipment will be immediately available for the management of anaphylaxis. Vaccination will be performed and the IMPs handled according to the relevant SOPs.

6.5 Minimising environmental contamination with genetically modified organisms (GMO)

The study will be performed in accordance with UK Genetically Modified Organisms (Contained Use) Regulations (2014). In order to minimise dissemination of the recombinant vectored vaccine virus into the environment, inoculation sites will be covered with a dressing after immunisation. This should absorb any virus that may leak out through the needle track. The dressing will be removed from the injection site after 30 minutes (+15/-5 minutes) and will be disposed as GMO waste by autoclaving.

7.3 Inclusion and exclusion criteria

This study will be conducted in healthy adults, with informed consent, who meet the following inclusion and exclusion criteria:

7.3.1 Inclusion Criteria

The volunteer must satisfy all the following criteria to be eligible for the study:

1. Healthy adults aged 18 to 50 years
2. Able and willing (in the Investigator’s opinion) to comply with all study requirements
3. Willing to allow the investigators to discuss the volunteer’s medical history with their General Practitioner
4. For females only, willingness to practice continuous effective contraception (see below) during the study and a negative pregnancy test on the day(s) of screening and vaccination
5. Agreement to refrain from blood donation during the course of the study
6. Provide written informed consent

7.3.2 Exclusion Criteria

The volunteer may not enter the study if any of the following apply:

1. Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment, or planned use during the study period
2. Prior receipt of an investigational vaccine likely to impact on interpretation of the trial data.
3. Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate
4. Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed)
5. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine
6. History of allergic reaction to Amynoglycoside antibiotics
7. Any history of hereditary angioedema, acquired angioedema, or idiopathic angioedema.
8. Any history of anaphylaxis in relation to vaccination
9. Pregnancy, lactation or willingness/intention to become pregnant during the study
10. History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ)
11. History of serious psychiatric condition likely to affect participation in the study
12. Bleeding disorder (e.g. factor deficiency, coagulopathy or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture
13. Any other serious chronic illness requiring hospital specialist supervision
14. Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week
15. Suspected or known injecting drug abuse in the 5 years preceding enrolment
16. Seropositive for hepatitis B surface antigen (HBsAg)
17. Seropositive for hepatitis C virus (antibodies to HCV)
18. Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis
19. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data
20. Inability of the study team to contact the volunteer's GP to confirm medical history and safety to participate

7.3.5 Criteria for postponement of vaccination
The following events constitute contraindications to administration of the vaccine at that point in time; if any one of these events occurs at the time scheduled for vaccination, the subject may be vaccinated at a later date, or withdrawn at the discretion of the Investigator.

- Acute disease at the time of vaccination. Acute disease is defined as the presence of a moderate or severe illness with or without fever. All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. temperature of ≤37.5°C/99.5°F.

- Temperature of >37.5°C (99.5°F) at the time of vaccination.

7.4 Compliance with Dosing Regime
All doses in this vaccine study will be administered by the Investigator and recorded in the CRF. The study medication will be at no time in the possession of the volunteer and compliance will not, therefore, be an issue.

7.5 Pregnancy
Should a volunteer become pregnant during the trial, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome. We will not routinely perform venepuncture in a pregnant volunteer.

8 TREATMENT OF TRIAL VOLUNTEERS
This section describes the clinical procedures for evaluating study participants and follow-up after administration of study vaccine.

8.1 Study procedures
All volunteers will have the same schedule of clinic attendances and procedures as indicated in the schedules of attendance (Table 4). All subjects will receive the ChAdOxi MERS-S vaccine, and undergo follow-up for a total of 26 weeks. The total volume of blood donated during the study will be mL. Additional visits or procedures may be performed at the discretion of the investigators, e.g., further medical history and physical examination, urine microscopy in the event of positive urinalysis or additional blood tests if clinically relevant.

8.2 Observations
Pulse, blood pressure and temperature will be measured at the time-points indicated in the schedule of procedures and may also be measured as part of a physical examination if indicated at other time-points.

8.3 Blood Tests and Urinalysis

Blood will be drawn for the following laboratory tests and processed:

1. At Oxford University Hospitals' NHS Trust, or Hammersmith Hospital using NHS standard procedures:
   - **Haematology;** Full Blood Count
   - **Biochemistry;** Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests (ALT, ALP, Bilirubin)
   - **Diagnostic serology;** HBsAg, HCV antibodies, HIV antibodies (specific consent will be gained prior to testing blood for these blood-borne viruses)
   - **Immunology;** Human Leukocyte Antigen (HLA) typing

Additional safety blood tests may be performed if clinically relevant at the discretion of the medically qualified investigators. These generally include, but are not limited to, AST, GGT and a coagulation screen.

2. At University of Oxford research laboratories:
   - **Exploratory Immunology;** Immunogenicity will be assessed by a variety of immunological assays. This may include antibodies to MERS Spike protein, ex vivo ELISpot assays for interferon gamma and flow cytometry assays, functional antibody assays and B cell analyses. Other exploratory immunological assays including cytokine analysis and other antibody assays, DNA analysis of genetic polymorphisms potentially relevant to vaccine immunogenicity and gene expression studies amongst others may be performed at the discretion of the Investigators.

3. **Urinalysis;** Urine will be tested for protein, blood and glucose at screening.
   For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (β-HCG) at screening and immediately prior to each vaccination.

Collaboration with other specialist laboratories in the UK, Europe and outside of Europe for further exploratory immunological tests may occur. This would involve the transfer of serum or plasma and/or PBMC to these laboratories, but these would remain anonymised. Informed consent for this will be gained from volunteers. Immunological assays will be conducted according to local SOPs.
Subjects will be informed that there may be leftover samples of their blood (after all testing for this study is completed), and that such samples may be stored indefinitely in the Oxford Vaccine Center Biobank for possible future research (exploratory immunology), including human DNA and RNA analyses to search for correlates of vaccine immunogenicity and efficacy. Subjects will be able to decide if they will permit such future use of any leftover samples. With the volunteers’ informed consent, any leftover cells, urine and serum/plasma will be frozen indefinitely for future ethically approved research studies of MERS specific or vaccine-related responses. If a subject elects not to permit this, all of that subject’s leftover samples will be discarded after the required period of storage to meet Good Clinical Practice (GCP) and regulatory requirements.

8.4 Study visits

8.4.2 Day 0: Enrolment and Vaccination Visit
Volunteers will not be considered enrolled in the study until they have received a vaccine. Before vaccination, the eligibility of the volunteer will be reviewed. Pulse, blood pressure and temperature will be observed and if necessary, a medical history and physical examination may be undertaken to determine need to postpone vaccination depending on criteria listed in section 7.3.5. Vaccinations will be administered as described below.

8.4.2.1 Vaccinations
Before each vaccination, the on-going eligibility of the volunteer will be reviewed. All vaccines will be administered intramuscularly according to SOP VC002 Vaccination as described above in section 6.4. The injection site will be covered with a sterile dressing and the volunteer will stay in the CCVTM for observation, in case of immediate adverse events. Observations will be taken 30 minutes after vaccination (+/- 5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes (+/- 10 minutes) before the volunteer leaves. An oral thermometer, tape measure and diary card (paper or electronic) will be given to each volunteer, with instructions on use, along with the emergency 24 hour telephone number to contact the on-call study physician if needed. Diary cards will collect information on the timing and severity of the following solicited AEs:

Table 3. Solicited AEs as collected on post vaccination diary cards

62
<table>
<thead>
<tr>
<th>Local solicited AEs</th>
<th>Systemic solicited AEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>Fever</td>
</tr>
<tr>
<td>Redness</td>
<td>Feverishness</td>
</tr>
<tr>
<td>Warmth</td>
<td>Joint pains</td>
</tr>
<tr>
<td>Itch</td>
<td>Muscle pains</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
</tr>
<tr>
<td></td>
<td>Malaise</td>
</tr>
</tbody>
</table>

Volunteers will be instructed on how to self-assess the severity of these AEs. There will also be space on the diary card to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

8.4.2.2 Sequence of Enrolment and Vaccination of Volunteers

For safety reasons, the first volunteer in Group 1 will be vaccinated ahead of any other volunteers and the profile of adverse events will be reviewed after 48 hours post vaccination. Provided there are no safety concerns, as assessed by the CI and the LSM, another 2 volunteers will be vaccinated at the same dose after at least 48 hours has elapsed following the first volunteer being vaccinated and at least 1 hour apart from each other. An independent safety review will be conducted by the LSM after vaccination of the first three volunteers. This review will include an assessment of the profile of adverse events and the results of safety blood tests at day 7 post vaccination. The CI and the LSM will be asked to provide the decision on whether to proceed with vaccinations of the remaining participants in group 1 and the first volunteer to receive the next incremental dose in group 2. If there are no safety concerns, the remaining volunteers in Group 1 and the first volunteer in group 2 may be vaccinated.

Enrolment of the first volunteer in Group 2 will only proceed if the CI and LSM assess the data from the first three vaccinees in Group 1 as indicating that it is safe to do so. The first subject in Group 2 will be vaccinated alone, and a 48 hour gap allowed before vaccinating further subjects in this group. Provided there are no safety concerns, as assessed by the CI and the LSM, another 2 volunteers will be vaccinated at the same dose after at least 48 hours has elapsed following the first volunteer being vaccinated and at least 1 hour apart from each other. An independent safety review will be conducted by the LSM after vaccination of the first three volunteers. This review will include an assessment of the profile of adverse events and the results of safety blood
tests at day 7 post vaccination. The CI and the LSM will be asked to provide the decision on whether to proceed with vaccinations of the remaining participants in group 2 and the first volunteer to receive the next incremental dose in group 3. If there are no safety concerns, the remaining volunteers in Group 2 and the first volunteer in group 3 may be vaccinated.

Enrolment of the first volunteer in Group 3 will only proceed if the CI and LSM assess the data from the first three vaccinees in Group 2 as indicating that it is safe to do so. The first subject in Group 3 will be vaccinated alone, and a 48 hour gap allowed before vaccinating further subjects in this group. Provided there are no safety concerns, as assessed by the CI and the LSM, another 2 volunteers will be vaccinated at the same dose after at least 48 hours has elapsed following the first volunteer being vaccinated and at least 1 hour apart from each other. An independent safety review will be conducted by the LSM after vaccination of the first three volunteers. This review will include an assessment of the profile of adverse events and the results of safety blood tests at day 7 post vaccination. The CI and the LSM will be asked to provide the decision on whether to proceed with vaccinations of the remaining participants in group 3. If there are no safety concerns, the remaining volunteers in Group 3 may be vaccinated.

8.4.3 Subsequent visits: days 2, 7, 14, 28, 56 and 182.
Follow-up visits will take place 48 hours (±24h), 7 days (± 2 days), 14 days (± 3 days), 28 days (± 3 days), 56 days (± 7 days) and 182 (± 14 days) after vaccination. Volunteers will be assessed for local and systemic adverse events, interim history, physical examination, review of diary cards (paper or electronic) and blood tests at these time points as detailed in the schedule of attendances. Blood will also be taken for exploratory immunology purposes.
If volunteers experience adverse events (laboratory or clinical), which the investigator (physician), CI and/or LSM determine necessary for further close observation, the volunteer may be admitted to an NHS hospital for observation and further medical management under the care of the Consultant on call.

Table 4. Schedule of attendances
<table>
<thead>
<tr>
<th>Attendance Number</th>
<th>1&lt;sup&gt;st&lt;/sup&gt;</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timeline** (days)</td>
<td>≤ 90</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>14</td>
<td>28</td>
<td>56</td>
<td>182</td>
</tr>
<tr>
<td>Time window (days)</td>
<td>± 1</td>
<td>± 2</td>
<td>± 3</td>
<td>± 3</td>
<td>± 7</td>
<td>± 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Review contraindications, inclusion and exclusion criteria</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccination</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital signs&lt;sup&gt;A&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ascertainment of adverse events</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Diary cards provided</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diary cards collected</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History, Physical Examination</td>
<td>X (X) (X) (X) (X) (X) (X) (X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochemistry®, Haematology (mL)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exploratory immunology&lt;sup&gt;E&lt;/sup&gt; (mL)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<td>Urinalysis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary HCG (women only)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA typing (mL)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg, HCV Ab, HIV serology (mL)</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood volume per visit</td>
<td>10</td>
<td>59</td>
<td>5</td>
<td>50</td>
<td>55</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cumulative blood volume%</td>
<td>10</td>
<td>69</td>
<td>74</td>
<td>79</td>
<td>12</td>
<td>9</td>
<td>184</td>
<td>234</td>
</tr>
</tbody>
</table>

S = screening visit; (X) = if considered necessary  
<sup>A</sup> Vital signs includes pulse, blood pressure and temperature;  
<sup>$</sup> = Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin and Liver function tests.  
<sup>E</sup> = Exploratory immunology includes antibodies to MERS S, ex vivo interferon-gamma ELISpot responses to MERS S

** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window.  
% Cumulative blood volume for Oxford volunteers if blood taken as per schedule, and excluding any repeat safety blood test that may be necessary.
9 ASSESSMENT OF SAFETY
Safety will be assessed by the frequency, incidence and nature of adverse events and serious adverse events arising during the study.

9.2 Foreseeable Adverse Reactions:
The foreseeable ARs following vaccination with ChAdOxi MERS-S include injection site pain, erythema, warmth, swelling, pruritus, myalgia, arthralgia, headache, fatigue, fever, feverishness, malaise and nausea.

9.3 Expected Serious Adverse Events
No serious adverse events are expected in this study.

9.6 Assessment of severity
The severity of clinical and laboratory adverse events will be assessed according to the scales in Tables 6-8, also described in the SOP VC027.

Table 6. Severity grading criteria for local adverse events.

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema at injection site*</td>
<td>1</td>
<td>&gt;3 - ≤50 mm</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;50 - ≤100 mm</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;100 mm</td>
</tr>
<tr>
<td>Swelling at injection site</td>
<td>1</td>
<td>&gt;3 - ≤20 mm</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;20 - ≤50 mm</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;50 mm</td>
</tr>
<tr>
<td>Ulceration/necrosis of skin at injection site</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Any</td>
</tr>
</tbody>
</table>

*erythema or swelling ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event.

Table 7. Severity grading criteria for physical observations

<table>
<thead>
<tr>
<th></th>
<th>Grade 1 (mild)</th>
<th>Grade 2 (moderate)</th>
<th>Grade 3 (severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (oral)</td>
<td>37.6°C - 38.0°C</td>
<td>38.1°C - 39.0°C</td>
<td>&gt;39.0°C</td>
</tr>
<tr>
<td>Tachycardia (bpm)*</td>
<td>101 - 115</td>
<td>116 - 130</td>
<td>&gt;130</td>
</tr>
<tr>
<td>Bradycardia (bpm)**</td>
<td>50 - 54</td>
<td>40 - 49</td>
<td>&lt;40</td>
</tr>
</tbody>
</table>
Table 8. Severity grading criteria for local and systemic AEs.

<table>
<thead>
<tr>
<th>GRADE 0</th>
<th>None: Symptom not experienced</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRADE 1</td>
<td>Mild: Short-lived or mild symptoms; medication may be required. No limitation to usual activity</td>
</tr>
<tr>
<td>GRADE 2</td>
<td>Moderate: Mild to moderate limitation in usual activity. Medication may be required.</td>
</tr>
<tr>
<td>GRADE 3</td>
<td>Severe: Considerable limitation in activity. Medication or medical attention required.</td>
</tr>
</tbody>
</table>

12. ETHICS

This is a descriptive safety study, where volunteers will be vaccinated with a single dose of ChAdOxi MERS-S. Twenty four (24) volunteers will be vaccinated in total. This sample size should allow an estimation to be made of the frequency and magnitude of outcome measures, rather than aiming to obtain statistical significance for differences between groups. Safety data will be presented according to frequency, severity and duration of adverse events.

Non-parametric tests will be used to determine differences in the primary immunogenicity outcome (ELISpot) data. A Wilcoxon signed rank test will permit testing of differences in responses between time points within a group and a Mann-Whitney U test will permit testing of differences in responses between different groups.

Sample Size Selection

This is a descriptive phase I first in human trial that will balance the safety of volunteers with the aims to assess the vaccine's safety profile and immunogenicity after selected doses of the vaccines. The primary dose comparison will be between Groups 1, 2 and 3, which will have 6-9 subjects each. MERS S-specific immunogenicity will be the key immunological readout assessed by a variety of immunological assays.
12.1 Declaration of Helsinki
The Investigators will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki.

12.2 Guidelines for Good Clinical Practice
The Investigators will ensure that this study is conducted in full conformity with the Good Clinical Practice (GCP).

12.3 Approvals
The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (PvEC), HPvA (where required), regulatory authorities (MHRA in the UK), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

12.5 Volunteer Confidentiality
All data will be anonymised: volunteer data will be identified by a unique study number in the CRF and database.

Example 4 - Immunohistopathology

We refer to Figure 10.

Intramuscular (IM)
A-B: Hematoxylin & Eosin
A: Vaccinated with unrelated Adenovirus; Lung: Normal - 100X
B: Chimp Adenovirus/MERS-CoV vaccine; Lung: Normal - 100X

Intranasal (IN)
C-D: Hematoxylin & Eosin
C: Vaccinated with unrelated Adenovirus; Lung: Multifocal, mild, interstitial infiltrates with lymphocytic perivascular cuffing - 100X.
D: Chimp Adenovirus/MERS-CoV vaccine; Lung: rare interstitial infiltrates with lymphocytic perivascular cuffing - 100X
Thus it is demonstrated that there is clearly no evidence of enhanced pathology or hypersensitivity in the MERS vaccinated groups.

**Example 5 - Safety Report**

This example presents safety and immunogenicity data for the MERS-CoV vaccine ChAdOxi MERS in both human volunteers (MERSooi phase I clinical trial) and camels, the principal animal reservoir for MERS coronavirus (MERS-CoV).

**Safety report for MERS001 phase I clinical trial**

MERSooi is a phase I clinical trial to determine the safety and immunogenicity of the candidate MERS-CoV vaccine ChAdOxi MERS in UK healthy adult volunteers. The study groups are shown in the table below.

**Table: Study groups for low \(5 \times 10^9\) viral particles (vp), medium \(2.5 \times 10^{10}\) vp and high \(5 \times 10^{10}\) vp dose vaccinations.**

<table>
<thead>
<tr>
<th>Group</th>
<th>ChAdOxi MERS dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=6)</td>
<td>(5 \times 10^9) vp</td>
</tr>
<tr>
<td>2 (n=9)</td>
<td>(2.5 \times 10^{10}) vp</td>
</tr>
<tr>
<td>3 (n=9)</td>
<td>(5 \times 10^{10}) vp</td>
</tr>
</tbody>
</table>

Safety reviews are required prior to each dose escalation of ChAdOxi MERS and prior to enrolment of the remaining volunteers in the group. The first review takes place after 3 volunteers in Group 1 have all received their single dose \(5 \times 10^9\) vp of ChAdOxi MERS and completed 7 days of follow-up post vaccine administration. The second review takes place after 3 volunteers in Group 2 have all received their single dose \(2.5 \times 10^{10}\) vp of ChAdOxi MERS and completed 7 days of follow-up post vaccine administration. At the time of writing, 3 volunteers have been enrolled in group 1 and 3 volunteers have been enrolled in group 2. All enrolled volunteers to date have reached their day 7 follow-up post vaccination. The table below shows the current progress of enrolment (completed follow-up visits in bold text).

**Table: Current progress of enrolment (completed follow-up visits in bold text).**

<table>
<thead>
<tr>
<th>Participant</th>
<th>DO</th>
<th>D2</th>
<th>D7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MERS-00101006</td>
<td>14/03/20</td>
<td>16/03/20</td>
<td>21/03/20</td>
</tr>
</tbody>
</table>
Adverse events (5 x 10^9 vp, low dose)

Solicited adverse events (AEs) after single dose of ChAdOxl MERS (5 x 10^9 vp):

The 7-day data from the E-diaries of the first 3 participants in Group 1, following their single vaccination, is shown in the table below (mild grade 1 AEs in yellow, moderate grade 2 AEs in orange). There have been no severe AEs and no SAEs following vaccine administration.

Volunteers MER-00101006 and MER-00101011 reported a short-lived mild pruritus at injection site at their 30 minutes observation in clinic. A Grade 1 mild redness at injection site (4mm) was observed at the 60 minutes review following vaccine administration in volunteer MER-00101011. All volunteers reported at least one systemic AE after vaccination. Systemic AEs were mild or moderate in nature and resolved within 24h.

Table: Solicited AEs self-reported (e-diaries) during 7 days after vaccine administration (5 x 10^9 vp ChAdOxl MERS)

<table>
<thead>
<tr>
<th>Participant</th>
<th>D14</th>
<th>D28</th>
<th>D56</th>
<th>D182</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MERS-00101004</td>
<td>20/03/20</td>
<td>22/03/20</td>
<td>27/03/20</td>
<td>12/04/20</td>
</tr>
<tr>
<td>MERS-ooioioio</td>
<td>12/04/20</td>
<td>14/04/20</td>
<td>19/04/20</td>
<td>12/09/2018</td>
</tr>
<tr>
<td>MERS-00101012</td>
<td>25/04/20</td>
<td>27/04/20</td>
<td>02/05/20</td>
<td>12/09/2018</td>
</tr>
<tr>
<td>MERS-ooioioio</td>
<td>02/05/20</td>
<td>04/05/20</td>
<td>09/05/20</td>
<td>12/09/2018</td>
</tr>
<tr>
<td>MERS-00101013</td>
<td>03/05/20</td>
<td>05/05/20</td>
<td>10/05/20</td>
<td>12/09/2018</td>
</tr>
<tr>
<td>Group 2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MERS-00101006</td>
<td>28/03/20</td>
<td>11/04/20</td>
<td>09/05/20</td>
<td>12/09/2018</td>
</tr>
<tr>
<td>MERS-00101004</td>
<td>03/04/20</td>
<td>17/04/20</td>
<td>15/05/20</td>
<td>18/09/2018</td>
</tr>
<tr>
<td>MERS-ooioioio</td>
<td>26/04/20</td>
<td>10/05/20</td>
<td>07/06/20</td>
<td>11/10/2018</td>
</tr>
<tr>
<td>MERS-00101012</td>
<td>09/05/20</td>
<td>23/05/20</td>
<td>20/06/20</td>
<td>24/10/2018</td>
</tr>
<tr>
<td>MERS-ooioioio</td>
<td>16/05/20</td>
<td>30/05/20</td>
<td>27/06/20</td>
<td>31/10/2018</td>
</tr>
<tr>
<td>MERS-00101013</td>
<td>17/05/20</td>
<td>31/05/20</td>
<td>28/06/20</td>
<td>01/11/2018</td>
</tr>
<tr>
<td>MER-00101006</td>
<td>0</td>
<td>2</td>
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<td>MER-00101004</td>
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<td>0</td>
</tr>
<tr>
<td>MER-00101011</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Pain | D | O | D1 | D2 | D3 | D4 | D5 | D6 |
| MER-00101006 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MER-00101004 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MER-00101011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Itch | D | O | D1 | D2 | D3 | D4 | D5 | D6 |
| MER-00101006 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MER-00101004 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MER-00101011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Warmth | D | O | D1 | D2 | D3 | D4 | D5 | D6 |
| MER-00101006 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MER-00101004 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MER-00101011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

<p>| Temperature | D | O | D1 | D2 | D3 | D4 | D5 | D6 |
| MER-00101006 | 36.2 | 36.3 | 36.1 | 36.1 | 35.8 | 36.8 |
| MER-00101004 | 36.5 | 36.4 | 36.4 | 36.4 | 36.7 | 36.8 |</p>
<table>
<thead>
<tr>
<th>Condition</th>
<th>D0</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthralgia</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Myalgia</td>
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</tr>
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<tr>
<td>Feverishness</td>
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<td>1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MER-00101011</td>
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<td>0</td>
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Unsolicited AEs after single dose of ChAdOxl MERS (5 x 10^9 vp)

Volunteer MER-00101006 reported mild (grade 1) 'Blocked nose', 'Running nose' and 'Cold symptoms' from D1 until Dio post vaccination. They also reported a mild and self-limited 'Loose bowel movement' episode on D5. Volunteer MER-00101011 reported mild and short-lived 'Back pain' on D1. They also reported mild 'Swollen glands / soreness when swallowing' symptoms from D2 until D6.

Serious Adverse Events

No SAEs have been reported until this date.

Clinical Observations

Observations are taken at each clinic visit from day 0 - 28. All three volunteers have been reviewed at the day 2 and day 7. Observations at all these time-points have been unremarkable.
Laboratory AEs
There are no Laboratory AEs reported to date.

5 Adverse events (2.5 x 10^10 vp, medium dose)

Solicited AEs after single dose of ChAdOxl MERS (2.5 x 10^10 vp)
The 7-day data from the E-diaries of the first 3 participants in Group 2, following their single vaccination, is shown below in table (mild grade 1 AEs in yellow, moderate grade 2 AEs in orange). There have been no severe solicited AEs and no SAEs following vaccine administration.

Volunteers MER-00101016 reported mild and short-lived warmth at injection site at their 30 min observation in clinic which completely resolved by their 60 min review. All volunteers reported at least one systemic AE after vaccination. Systemic AEs were mild or moderate in nature and resolved within 5 days.

Table: Solicited AEs self-reported (e-diaries) during 7 days after vaccine administration (2.5 x 10^10 vp ChAdOxl MERS).

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Unsolicited AEs after single dose of ChAdOxl MERS (2.5 x 10^{10} vp)

Volunteer MER-00101012 reported mild (grade 1) Photophobia and Earache on D1 which resolved within 24h. Volunteer MER-00101010 reported mild Lack of appetite on D1 and moderate ‘Diarrhoea’ on D2. They remained asymptomatic on D3, but then reported gastroenteritis symptoms starting with mild Nausea, Malaise & Vomiting on D4 which got progressively worse until D7 when they experienced severe (grade 3) Nausea, Abdominal Pain, Vomiting and Diarrhoea. Volunteer called their GP who prescribed anti-emetics and advised increased fluids intake. Contacted over the phone on D8 (date of this report) and volunteer reported significant improvement on frequency of Diarrhoeal episodes and Nausea, Vomiting and Abdominal Pain have now ceased. Volunteer MER-00101013 reported mild Light-headedness on D2, which resolved within 24h.

Serious Adverse Events

No SAEs have been reported until this date.

Clinical Observations

Observations are taken at each clinic visit throughout the trial. All three volunteers have been reviewed at days 2 and 7 following vaccination. Volunteer MERS-00101010 had a grade 1 (mild) Bradycardia with a heart rate of 53 bpm at 60 min post vaccine administration and 54 bpm at D7 (baseline heart rate = 67 bpm). Volunteer was well and asymptomatic. There are no further significant changes in their clinical observations.

Laboratory AEs

Volunteer MERS-00101012 had a grade 2 (moderate) microcytic anaemia (Hb = 101 g/L, MCV = 75.2 fl on D2; Hb = 104 g/L, MCV 74.3 fl on D7). This represents a non-significant change from their baseline microcytic anaemia likely due to iron deficiency (Hb = 105 g/L, MCV = 75.7 on Do).

Volunteer MERS-00101013 had a grade 2 (moderate) normocytic anaemia (Hb = 104 g/L, MCV = 85 fl on D7). This represents a non-significant change from their baseline normocytic anaemia (Hb = 106 g/L, MCV = 86 on Do). This volunteer also had a grade
(mild) Neutropenia \((N = 1.4 \times 10^9/\text{L})\). All safety blood tests will be repeated at D28, as per protocol.

**Adverse events** \((5 \times 10^{10} \text{ vp, high dose})\)

The first Group 3 volunteer is scheduled to be vaccinated with the highest dose of ChAdOxi MERS \((5 \times 10^{10} \text{ vp})\) on the 14th May 2018, pending the review of the safety report data.

**Example 6: MERS001 Interim analysis of immunology data.**

**Methods**

**ELISPOT**

The Enzyme-Linked ImmunoSpot (ELISPOT) assay is a widely used method for monitoring cellular immune responses. Herein it is used to measure interferon gamma (IFN\( \gamma \)) produced by T cells (activated in volunteers in response to vaccination) responding to MERS CoV peptides in culture. Ex vivo (18-h stimulation) ELISPOT assays were performed using Multiscreen IP ELISPOT plates (Millipore), human IFN\( \gamma \)-SA-ALP antibody kits (Mabtech) and BCIP NBT-plus chromogenic substrate (Moss Inc). Cells were cultured in RPMI (Sigma) containing 10% heat-inactivated, sterile-filtered fetal calf serum, previously screened for low reactivity (Labtech International). Antigens were tested in duplicate with 250,000 PBMC added to each well of the ex vivo ELISPOT plate. Peptides were 20 amino acids in length, overlapping by 10 amino acids (Neopeptide), assayed in 13 pools of peptides at 10 mg/ml (tables below).

Tables: MERS-CoV Si peptide pools for ELISPOT assay. tPA indicates peptides covering the leader sequence/secretory tissue plasminogen activator amino acid sequence which is fused to the N-terminus of the MERS-CoV spike protein antigen. Responses to tPA are not included in the ELISPOT response SFC counts.

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**Peptide Sequences:**

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- Peptide 2: IHARFPvRIHSVFLLM LFVANYSQ DVKQFAN ATYHTPATDCSDGNY
- Peptide 3: IHARFPvRIHSVFLLM LFVANYSQ DVKQFAN ATYHTPATDCSDGNY
- Peptide 4: IHARFPvRIHSVFLLM LFVANYSQ DVKQFAN ATYHTPATDCSDGNY
- Peptide 5: IHARFPvRIHSVFLLM LFVANYSQ DVKQFAN ATYHTPATDCSDGNY
- Peptide 6: IHARFPvRIHSVFLLM LFVANYSQ DVKQFAN ATYHTPATDCSDGNY

Each peptide pool contains a set of peptides with varying sequences, designed to test for binding or other biological responses.
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Responses were averaged across triplicates, responses in unstimulated (negative control) wells were subtracted and then responses in individual pools were summed. Staphylococcal enzyme B (0.04 mg/ml) and phytohaemmagglutinin-L (20 ug/ml) were used as a positive control. Plates were counted using an AID automated ELISPOT counter (AID Diagnostika GmbH, algorithm C), using identical settings for all plates and counts were adjusted only to remove artefacts. Responses to the negative control were always <80 spot forming cells (SFC) per million peripheral blood mononuclear cells (PBMC) and >800 SFC for the positive control. Results are shown in figure 11.

Results

T cell responses to ChAdOxl MERS (5 x 10⁹ vp, low dose)

ELISPOP responses as spot forming cells (IFN/producing activated T cells) per million PBMCs were determined for each volunteer pre vaccination, and at 14 and 28 days post vaccination. Results are displayed in figure 11.

Figure 11 shows spot forming cells (T cells activated in response to MERS CoV peptides) per million peripheral blood mononuclear cells (the rest of the cells in a given volume of blood). Group 1 (n=3) volunteers were vaccinated with a single dose of ChAdOxl MERS (5 x 10⁹ vp, low dose). Data points indicate the summed response for each volunteer to all pools of peptides in the MERS S1 protein component of the ChAdOxl MERS vaccine. All volunteers showed a significant increase in response at day 14 after vaccination (p<0.05, Kruskall-Wallis test).

Responses to MERS S1 protein peptide pools prior to vaccination were low, with a median response of 85 SFC per million PBMC, increasing to a median of 1411 SFC at day 14 in the low dose group. Individual responses are tabulated below.

Individual ELISPOT responses (mean per patient) to MERS CoV peptide pools:

<table>
<thead>
<tr>
<th>Patient</th>
<th>Group</th>
<th>Dose (v.p.)</th>
<th>DO</th>
<th>D14</th>
<th>D28</th>
<th>D56</th>
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<td>85</td>
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</tbody>
</table>

SFC per million PBMC
X indicates data due in the next two weeks.

**Example 7: Camel study**

**Methods**

*ChAdOx1 MERS*

Average from 3 vials batch 01N17-01: $1.74 \times 10^{11}$ vp/mL and $1.61 \times 10^9$ ifu/mL.

*Commercial MERS-CoV S protein specific ELISA from Euroimmun*

Antibody responses in vaccinated camels were evaluated using a commercial camel MERS-CoV S protein specific ELISA kit from Euroimmun (1). The results of this kit are presented as ratios of optical density (OD) of each serum sample over the OD of a provided positive calibrator, the positive cut-off is 1.1 following manufacturers recommendation.

*In house MERS-CoV S protein specific ELISA*

Enzyme-linked immunosorbent assay (ELISA) is an assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. Herein it is used to measure serum antibody responses to the MERS-CoV S protein. ELISA plates were coated with 2 µg/ml capturing antigen (S protein recombinant from ATGen Co. Ltd., CA, USA, provided as part of a collaboration with the International Vaccine Institute of Seoul National University, Seoul, Korea), and standard endpoint ELISA protocol was followed, as previously described (2). Sera were prepared in a 10-fold serial dilution in PBS/T and then 50 µl were plated in duplicate wells. Anti-camel IgG conjugated to alkaline phosphatase (Sigma) and PNPP tablet (20 mg p-nitrophenylphosphate, SIGMA) substrate were used in the assay.

*Virus neutralisation assay*

Induction of virus-neutralising antibodies was confirmed according to previously published protocols (3, 4). Serum samples were tested for their capacity to neutralize MERS-CoV in tissue culture infections by using 100 50% tissue culture infective doses (TCID$_{50}$) in Huh-7 cells. Virus neutralisation titre is calculated as the reciprocal of the serum dilution that neutralizes the virus infection.
**Experimental animals**

Camels were purchased from rural markets in the north of Saudi Arabia and maintained at a camel research farm, managed by the Saudi Ministry of Water, Environment, and Agriculture (MEWA). Animal maintenance and procedures were in accordance with King Abdullah International Medical Research Centre (KAIMRC, Riyadh, Kingdom of Saudi Arabia) Institutional Animal Care and Use Committee (IACUC) recommendations, under the regulation of MEWA. Project designation is KAIMRC research project RC16-093.

**Pilot study:** Nine naïve calves, with Euroimmun ELISA ratios below the positive cut-off value of 1.1 were divided into 3 groups (n=3 per group). In the control group, two calves received phosphate buffered saline (PBS) and one calf received lxio\(^9\) Infectious Unit (IU) of ChAdOxi-eGFP, intramuscularly as a vector control. The second group comprised 3 young calves of 8-10 months old that received lxio\(^9\) IU of ChAdOxi MERS vaccine via the intramuscular route. The third group comprised 3 older calves of 18-24 months old that received same vaccine regimen as the second group.

**Main study:** Ten naïve calves were divided into 2 groups (n=5 per group). Group 1 naïve (G01-N) received control injections of PBS (n=3) or lxio\(^9\) ChAdOxi-eGFP (n=2) and group 2 naïve (G02-N) received vaccine injections of lxio\(^9\) ChAdOxi MERS (n=5). All vaccination was performed via the intramuscular route. Serum samples were collected pre-immunisation (day 0) and at 7, 14, 21, and 28 days post immunisation (d.p.i.). Two calves were boosted with a second dose at 45 d.p.i. and serum samples were collected post-boost (p.b.) at 49, 56, 63, and 70 d.p.i. In addition, 10 seropositive camels were divided into 2 groups (n=5 per group); where Group 1 seropositive (G01-S) group received control injections of PBS (n=3) or lxio\(^9\) ChAdOxi-eGFP (n=2) and Group 2 seropositive (G02-S) received lxio\(^9\) ChAdOxi MERS (n=5). Serum samples were collected pre-immunisation (day 0) and at 7, 14, 21, 28, and 56 d.p.i.

Below is age information for the camels in the main study. The age of naïve camels are very young. The seropositive camels vary from 1 to 3 years. See the table below - the top set is G01, lower set G02.

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<tr>
<th>Set</th>
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<td>6-12 m</td>
</tr>
<tr>
<td>Seropositive</td>
<td>6-12 m</td>
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</table>
Seropositive | i8-24m
---|---
Seropositive | 8 months
Seropositive | 18-24 ITL
Seropositive | 6-12 m

Naive | 6-12 m
Naive | 6-12 m
Naive | 6-12 m
Naive | 6-12 m
Naive | 6-12 m
Seropositive | 6-12 m
Seropositive | 2-5 - 3 years
Seropositive | 2-5 - 3 years
Seropositive | 2-5 - 3 years
Seropositive | 2-5 - 3 years

**Vaccination**
Camels received $1xio^2$ Infectious Units (IU) of ChAdOxi-MERS or ChAdOxi-eGFP (as a vector control) per vaccination via the intramuscular route.

**Statistical analysis**
GraphPad Prism (GraphPad software) was used for statistical analysis and to plot data.

**Ethics statement**
All animal procedures were performed in accordance with the terms of the UK Animals (Scientific Procedures) Act (ASPA) and were approved by the University of Oxford Animal Care and Ethical Review Committee. Animal maintenance and procedures were in accordance with King Abdullah International Medical Research Centre (KAIMRC, Riyadh, Kingdom of Saudi Arabia) Institutional Animal Care and Use Committee (IACUC) recommendations, under the regulation of the Saudi Ministry of Water, Environment, and Agriculture (MEWA).

**Results**

**Commercial ELISA results - Pilot study**
Antibody responses following vaccination with ChAdOxi-MERS were evaluated using the commercial ELISA kit from Euroimmun.
Data are displayed in figure 12: Groups of calves (n=3) in vaccinated groups received \(1 \times 10^9\) IU of ChAdOxi-MERS. The control group received either phosphate buffered saline (PBS, n=2) and or \(1 \times 10^9\) Infectious Unit (IU) of ChAdOxi—eGFP (vector control, n=i). Young calves were 8-10 months old. Older calves were 18-24 months old. Data points show the ratio of the optical density (OD) of test samples over the manufacturer's positive calibrator OD. All vaccination was performed via the intramuscular route. Data points represent individual camels with the mean shown as a horizontal line.

Antibody responses indicated by ELISA ratios greater than 1.1 were seen from day 7 in the older calves (18-24 months) following a single dose of ChAdOxi-MERS. Younger calves did not respond to the single dose of vaccine to the same extent, with only one animal producing a positive result from day 56. There is a statistically significant difference between the ELISA ratios of the younger and older calves (2 way ANOVA, \(p < 0.0001\)).

**In house ELISA results - Pilot study**

An in house ELISA was developed to allow calculation of endpoint titres.

Samples from figure 12 were analysed using the in house ELISA, this analysis is shown in figure 13: samples and groups are as figure 12, except data are not available for days 7-21 therefore are not included. ELISA plates were coated with Si recombinant protein (from ATGen Co. Ltd., CA, USA, provided as part of a collaboration with the International Vaccine Institute of Seoul National University, Seoul, Korea), and standard endpoint ELISA protocol was followed, as previously described (1). Control animals were all negative. Data points represent individual camels with the mean shown as a horizontal line.

There is a statistically significant difference between the end point titres of the younger and older calves (2 way ANOVA, \(p < 0.05\)).

**Virus Neutralisation Assay - Pilot study**

A virus neutralisation assay was used to assess the ability of vaccine induced antibodies to neutralise MERS CoV infection in tissue culture.
Results are displayed in figure 14: virus neutralisation titre (VNT) was calculated for camel serum samples collected at 0 and 28 d.p.i. Serum samples were tested for their ability to neutralise MERS CoV infection in tissue culture by using 100 50% tissue culture infective doses (TCID<sub>50</sub>) in HuH-7 cells. Virus neutralisation titre is calculated as the reciprocal of the serum dilution that neutralizes the virus infection. Data points represent individual camels with the mean shown as a horizontal line.

Sera from older calves neutralised MERS CoV infection at much higher dilutions (mean 1 in 655 dilution) than sera from younger calves. Only serum from one of the younger calves was able to neutralise MERS CoV infection at a dilution of 1 in 16. This indicates the presence of effective neutralising antibodies in the older calves compared to the younger cohort. There is a statistically significant difference between the neutralisation titres of the younger and older calves (2 way ANOVA, p <0.05).

Commercial ELISA results - Main study
Antibody responses following vaccination with ChAdOxi-MERS were evaluated using the commercial ELISA kit from Euroimmun. Data are displayed in figure 15 and figure 16.

Referring to Figure 15, 10 naive (negative for MERS antibodies) calves were divided into 2 groups (n=5 per group); where G01-N group received control injections of PBS (n=3) or Lxio<sup>9</sup> ChAdOxi-eGFP as a vector control (n=2) and G02-N received vaccine injections of Lxio<sup>9</sup> ChAdOxi MERS (n=5) intramuscularly. Serum samples were collected pre-immunisation (0 d.p.i.) and at 7, 14, 21, and 28 days post immunisation (d.p.i.). Two calves were boosted with a second dose at 45 d.p.i. and serum samples were collected weekly post-boost (pb) at 40, 56, 63, and 70 d.p.i. Samples were analysed using the commercial kit from Euroimmun. Data points represent individual camels with the mean shown as a horizontal line.

Responses above the manufacturers recommended cut-off were measurable only in animals that received the boost vaccination at day 45. There is a statistically significant difference between the ELISA ratios of the younger and older calves post boost (2 way ANOVA, p <0.0001).

Referring to figure 16, 10 camels that tested positive for the presence of anti MERS antibodies (seropositive) were divided into 2 groups (n=5); where G01-S group received control injections of PBS (n=3) or Lxio<sup>9</sup> ChAdOxi-eGFP (n=2) and G02-S received
vaccine injections of Lxio ChAdOxi MERS (n=5) intramuscularly. Serum samples were collected pre-immunisation (0-0.1 day post-immunisation) and post-immunisation at 7, 14, 21, 28, and 56 days post-immunisation (d.p.i.). Samples were analysed using the commercial kit from Euroimmun that measures antibodies against the Si protein. Data points represent individual camels with the mean shown as a horizontal line.

Responses above the manufacturer’s recommended cut-off were measurable in most of the control animals and in all of the vaccinated camels with the exception of one animal at day 7. Responses in the vaccinated animals were generally higher than in the control animals, indicating that vaccination with ChAdOxi MERS can boost responses to the Si protein in animals previously exposed to MERS-CoV. There is a statistically significant difference between the ELISA ratios of the control and vaccinated calves (2-way ANOVA, p < 0.0001).

In house ELISA results - Main study

Antibody responses following vaccination with ChAdOxi-MERS were evaluated using an in-house ELISA. Data are displayed in figure 17 and figure 18.

Referring to figure 17, 10 naïve (negative for MERS antibodies) calves were divided into 2 groups (n=5 per group); where G01-N group received control injections of PBS (n=3) or Lxio ChAdOxi-eGFP as a vector control (n=2) and G02-N received vaccine injections of Lxio ChAdOxi MERS (n=5) intramuscularly. Serum samples were collected pre-immunisation (0 days post-immunisation) and at 7, 14, 21, and 28 days post-immunisation (d.p.i.). Two calves were boosted with a second dose at 45 days post-immunisation (d.p.i.) and serum samples were collected post-boost (pb) at 49, 56, 63, and 70 days post-immunisation (d.p.i.). Samples were analysed using an in-house end point titre ELISA that measures response against the Si protein. These ELISA data are for the same serum samples as in figure 15. Data points represent individual camels with the mean shown as a horizontal line.

Responses were measurable only in animals that received the boost vaccination at day 45.

Referring to figure 18, 10 camels that tested positive for the presence of anti MERS antibodies (seropositive) were divided into 2 groups (n=5); where G01-S group received control injections of PBS (n=3) or Lxio ChAdOxi-eGFP (n=2) and G02-S received vaccine injections of Lxio ChAdOxi MERS (n=5) intramuscularly. Serum samples were collected pre-immunisation (0 days post-immunisation) and post-immunisation at 7, 14, 21, 28, and 56 days post-immunisation (d.p.i.).
d.p.i. Samples were analysed using an in house end point titre ELISA that measures responses against the S1 protein. These ELISA data are for the same serum samples as in figure 16. Data points represent individual camels with the mean shown as a horizontal line.

Responses were measurable in all of the control animals and in all of the vaccinated camels at all time-points. Responses in the vaccinated animals were generally higher than in the control animals, indicating that vaccination with ChAdOxi MERS can boost responses to the S1 protein in animals previously exposed to MERS CoV. These differences were not statistically significant.

**Summary**

The MERS001 phase I clinical trial has demonstrated safety at (5 x 10⁹ and 2.5 x 10¹⁰ vp (low and medium doses) and immunogenicity at 5 x 10⁹ vp. Adverse events following vaccine administration have been mild or moderate in nature and self-resolving at both doses. T cell ELISPOT responses have been relatively low but significant at the low dose.

In camels, antibody responses were detectable by both commercial and in house ELISAs in older calves following a single vaccination and in younger animals that received a boost at 45 dpi. In seropositive animals ChAdOxi MERS boosted pre-existing antibody responses. Antibodies generated by ChAdOxi MERS were able to neutralise MERS CoV in tissue culture. Thus it can be appreciated that there are no adverse effects, and only very mild side effects.

**References to examples 5 to 7**


Although illustrative embodiments of the invention have been disclosed in detail herein, with reference to the accompanying drawings, it is understood that the invention is not limited to those precise embodiments and that various changes and modifications can be effected therein by one skilled in the art without departing from the scope of the invention as defined by the appended claims and their equivalents.
| SEQ ID NO: 1 | spike protein GenBank accession number AHX71946.1 |
| SEQ ID NO: 2 | ChAdOx2: Viral vector based on Chimpanzee adenovirus C68 |
| SEQ ID NO: 3 | nucleotide sequence codon optimised spike protein without tPA leader |
| SEQ ID NO: 4 | nucleotide sequence codon optimised spike protein with tPA leader |
| SEQ ID NO: 5 | tPA amino acid sequence (P->A mutant) |
| SEQ ID NO: 6 | tPA amino acid sequence (naturally occurring P) |
| SEQ ID NO: 7 | tPA amino acid sequence SEQ ID NO: 5 without ‘RR’ |
| SEQ ID NO: 8 | tPA amino acid sequence SEQ ID NO: 6 without ‘RR’ |
| SEQ ID NO: 9 | nucleotide sequence encoding tPA SEQ ID NO: 5, which has been codon optimised for human codon usage |

**SEQUENCE LISTING**

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CLAIMS

1. A composition comprising a viral vector, the viral vector comprising nucleic acid having a polynucleotide sequence encoding the spike protein from the middle eastern respiratory syndrome coronavirus (MERS-CoV), characterised in that said viral vector is an adenovirus based vector.

2. A composition according to claim 1 wherein said adenovirus based vector is ChAdOx 1.

3. A composition according to claim 1 or claim 2 wherein said spike protein comprises the receptor binding domains (RBDs).

4. A composition according to any of claims 1 to 3 wherein said spike protein is full length spike protein.

5. A composition according to any preceding claim wherein said spike protein is present as a fusion with the tissue plasminogen activator (tPA) sequence in the order N-terminus - tPA - spike protein - C-terminus.

6. A composition according to claim 5 wherein said tPA has the amino acid sequence SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

7. A composition according to any preceding claim wherein said spike protein has the amino acid sequence SEQ ID NO: 1.

8. A composition according to any preceding claim wherein said polynucleotide sequence comprises the sequence of SEQ ID NO: 3 or SEQ ID NO: 4.

9. A composition according to any of claims 2 to 8 wherein said viral vector sequence is as in ECACC accession number 12052403.

10. A composition according to any preceding claim wherein administration of a single dose of said composition to a mammalian subject induces protective immunity in said subject.
11. A composition according to any preceding claim for induction of an immune response against MERS-CoV.

12. A composition according to any preceding claim for induction of an immune response against MERS-CoV, wherein a single dose of said composition is administered.

13. A composition according to any preceding claim for induction of an immune response against MERS-CoV, wherein said composition is administered once.

14. A composition according to claim 13, wherein said composition is administered once per 6 months.

15. A composition according to claim 13, wherein said composition is administered once per 12 months.

16. A composition according to any preceding claim for preventing MERS-CoV infection.

17. A composition according to any preceding claim for preventing MERS-CoV infection, wherein a single dose of said composition is administered.

18. A composition according to any preceding claim for preventing MERS-CoV infection, wherein said composition is administered once.

19. A composition according to claim 18, wherein said composition is administered once per 6 months.

20. A composition according to claim 18, wherein said composition is administered once per 12 months.

21. Use of a composition according to any preceding claim in medicine.

22. Use of a composition according to any preceding claim in the preparation of a medicament for prevention of MERS-CoV infection.
23. A method of inducing an immune response against middle eastern respiratory syndrome coronaviruses (MERS-CoV) in a mammalian subject, the method comprising administering a composition according to any preceding claim to said subject.

24. A method according to claim 23 wherein a single dose of said composition is administered to said subject.

25. A method according to claim 23 or 24 wherein said composition is administered once.

26. A method according to claim 25 wherein said composition is administered once per 6 months.

27. A method according to claim 25 wherein said composition is administered once per 12 months.

28. A method according to any of claims 23 to 27 wherein said composition is administered by a route of administration selected from a group consisting of subcutaneous, intranasal, aerosol, nebuliser, intradermal and intramuscular.

29. A method according to claim 28 wherein said administration is intramuscular.
FIGURE 4 continued

D  MERS-S peptide (IC S)

% Cytokines^+ splenic CD8^+ T cells

IFN-γ  TNF-α  IL-2  IL-17
Cytokine

E  E3 MVA-specific peptide

% Cytokines^+ splenic CD8^+ T cells

IFN-γ  TNF-α  IL-2  IL-17
Cytokine

F  F2(G) MVA-specific peptide

% Cytokines^+ splenic CD8^+ T cells

IFN-γ  TNF-α  IL-2  IL-17
Cytokine
FIGURE 5

A

Endpoint titre (Log_{10})

mh5-MVA MERS
F11-MVA MERS
21 d.p.i. (Post-prime)
42 d.p.i. (Post-boost)

B

IC90 neutralisation titre (Log_{10})

mh5-MVA MERS
F11-MVA MERS

p = 0.9372

C

IFNγ SFU/10^6 splenocytes

mh5-MVA/ mh5-MVA
F11-MVA/ F11-MVA

p = 0.0043
FIGURE 5 continued

D

E3 MVA-specific peptide

% Cytokines+ splenocytes

Cytokines

E

F2(G) MVA-specific peptide

% Cytokines+ splenocytes

Cytokines
FIGURE 10

A

B

C

D

FIGURE 11

B

ELISPOT response to ChAdOx1 MERS

SFC per 10^6 PBMC

10000

1000

100

10

D0

D14

D28

Time Point (Days)

*, p<0.05 comparing ELISPOT response at D14 to D0. Kruskall-Wallis test.
FIGURE 14

- Control
- Vaccinated (young calves)
- Vaccinated (older calves)

Viral neutralisation titre

Days post immunisation (priming dose)

FIGURE 15

Naive camels

- G01-N (control)
- G02-N (vaccinated)

Euroimmun ELISA Ratio

Days post immunisation (priming dose)
FIGURE 18

Seropositive camels

○ G01-S (control)
● G02-S (vaccinated)

Endpoint titre (Log_{10})

Days post immunisation (priming dose)
A. CLASSIFICATION OF SUBJECT MATTER
INVENTION:
A61K39/215 A61P11/00 A61P31/14

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols):
A61K C12N A61P

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used):
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Relevant to claim No.</th>
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<td>CN 105 543 248 A (INST FOR VI RAL DISEASE CONTROL AND PREVENTION CHINESE CENTER FOR DISEASE) 4 May 2016 (2016-05-04) the whole document</td>
<td>1,3-8, 10-21, 23,26-29</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search: 20 September 2018

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Date of mailing of the international search report: 09/10/2018

Authorized officer: Domingues, Helena
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. [ ] forming part of the international application as filed:
      [ ] in the form of an Annex C/ST.25 text file.
      [ ] on paper or in the form of an image file.
   b. [ ] furnished together with the international application under PCT Rule 13ter.1 (a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:
      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1 (a)).
      [ ] on paper or in the form of an image file (Rule 13ter.1 (b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
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<td>XIAOJUAN GUO ET AL: &quot;Systemic and mucosal immunity in mice elicited by a single e immuni zation with human adenovirus type 5 or 41 vector-based vaccines carrying the spike protein of Mddl e East respiratory syndrome coronavirus&quot;, IMMUNOLOGY, vol. 145, no. 4, 21 April 2015 (2015-04-21), pages 476-484, XP055499409, GB</td>
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<td>GEORGE M. WARI MWE ET AL: &quot;Chimpanzee Adenovirus Vaccine Protects against Rift Valley Fever&quot;, SCIENTIFIC REPORTS, vol. 6, no. 1, 5 February 2016 (2016-02-05), XP055499794, DOI: 10.1038/srep20617, the whole document</td>
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