Safety Platform for Emergency vACcines

D2.4 Preliminary guidance on safety data collection for COVID-19 vaccine safety

Work Package: WP2 Standards and tools

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Description of the deliverable	This deliverable provides guidance on the collection of safety data for COVID-19 vaccine candidates. This includes a brief discussion of the types of safety data to collect in trials, a sample memory aid template for collection of solicited local and systemic reactions with guidance re: data analysis and presentation, a literature search on enhanced disease and a report of the consensus conference on enhanced disease following immunization.
Key words	Enhanced disease, local reactions, systemic reactions, AEFI, AESI, memory aid, Brighton Collaboration case definitions

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DEFINITIONS & ACRONYMS

ACE2	Angiotensin-converting enzyme 2
ADE	Antibody disease enhancement
AE	Adverse event
AEFI	Adverse event following immunization
AESI	Adverse event of special interest
ARDS	Acute respiratory distress syndrome
B/HPIV3	Bovine/human parainfluenza virus type 3
BC	Brighton Collaboration
BPL	β-Propiolactone
BtCoV	Bat coronavirus
CEPI	Coalition for Epidemic Preparedness Innovations
CNS	Central nervous system
CoV	Coronavirus
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRISPR	Clustered regularly interspaced short palindromic repeats
DNA	Deoxyribonucleic acid
DPP4	Dipeptidyl peptidase-4
hACE2	Human ACE2 receptor
HBs	Hepatitis B surface antigen
hDPP4	Human DPP4
IHC	Immunohistochemistry
MERS CoV	Middle East respiratory syndrome coronavirus
mRNA	Messenger RNA
MVA	Modified Vaccinia Virus Ankara
NHP	Non-human primate
Non-SPF	Non-specific pathogen free
NTD	N terminal domain
RAG1	Recombination activating gene 1
RBD	Receptor binding domain
rMVA	Recombinant modified vaccinia virus Ankara
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
SARS-CoV-1	Severe acute respiratory syndrome coronavirus 1
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SPEAC	Safety Platform for Emergency vACcines
TCR	T-cell receptor
Tg	Transgenic
Th1	T-helper cell type 1
Th2	T-helper cell type 2
VSV	Vesicular stomatitis virus
WHO	World Health Organization

EXECUTIVE SUMMARY

A novel coronavirus (CoV), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), emerged in late 2019 in Wuhan, China and has since spread as a global pandemic. Safe and effective vaccines are urgently needed to reduce the significant morbidity and mortality of Coronavirus Disease 2019 (COVID-19) and ease the major economic impact. There has been an unprecedented rapid response by vaccine developers with >140 vaccine candidates in preclinical development and >20 having reached clinical trials. However, a major challenge during rapid development is to avoid safety issues both by thoughtful vaccine design and by thorough safety evaluation in a timely manner. This SO2 D2.4 deliverable was meant to summarize recommendations for vaccine safety data collection, CRF, presentation and analysis for clinical trials of CEPI funded vaccine candidates targeting COVID-19.

SPEAC has responded to this challenge by summarizing recommendations by the Brighton Collaboration and SPEAC project for vaccine safety data collection, CRF, presentation and analysis for clinical trials of CEPI funded vaccine candidates targeting COVID-19 with sample CRF presented in Appendix 1. In response to a request by CEPI to provide comments to the 19April version of the WHO Solidarity Protocol, SPEAC outlined the safety evaluations that should be included for trials of COVID-19 vaccine candidates with input from some Meta-DSMB members. As an example, we provided a memory aid for collection of solicited local and systemic reactions following immunization included as Appendix 2. A guide for presentation and analysis of these solicited local and systemic reaction data is included as Appendix 3 using the Brighton Collaboration case definitions as a reference where available. We also reviewed the information about enhanced disease following immunization with prior coronavirus vaccine candidates for SARS and MERS, establishing an initial literature search to gather the available information on this topic and then with the support of CEPI (Appendix 4), and organized a virtual 2-day consensus meeting on March 12 and 13, 2020 to review the preclinical data with a panel of experts. This meeting led to consensus recommendations for developers with the input of regulators attending the meeting that has been published and is included as Appendix 5.

1. Background

3. The need for standardization

A novel coronavirus (CoV), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), emerged in late 2019 in Wuhan, China and has since spread as a global pandemic. Safe and effective vaccines are urgently needed to reduce the significant morbidity and mortality of Coronavirus Disease 2019 (COVID-19) and ease the major economic impact. There has been an unprecedented rapid response by vaccine developers with >140 vaccine candidates in preclinical development and >20 having reached clinical trials according to WHO's 28JUL2020 Landscape of COVID-19 Candidate Vaccines[1]. However, a major challenge during rapid development is to avoid safety issues both by thoughtful vaccine design and by thorough and harmonized evaluation in a timely manner.

To maximize the value of vaccine safety data in clinical trials, it is essential to have standardized approaches to their data collection, presentation and analysis.

Without globally accepted standard case definitions for assessing adverse events following immunization (AEFIs), it is difficult, if not impossible, to compare safety data across studies with any validity. Global standardization might enable comparability of vaccine safety data collected from clinical trials, surveillance systems, individual case reports, and retrospective epidemiologic studies.

In the CEPI-funded vaccine development programs, the CEPI-funded developers are the sponsors, are responsible for safety monitoring of their products and have the responsibility to comply with regulatory requirements. Since CEPI funds several developers that develop vaccines for the same target but using different vaccines and platforms, harmonization of safety monitoring is essential to allow for meaningful analysis and interpretation of the safety profiles of CEPI-funded vaccines.

CEPI has contracted with the Brighton Collaboration, through the Task Force for Global Health, to harmonize the safety assessment of CEPI-funded vaccines via its Safety Platform for Emergency vACcines (SPEAC) Project.

4. Guidance from regulators

In a June 22, 2020 teleconference of global regulators convened jointly by EMA and US FDA under the auspices of ICMRA, participants discussed preclinical and clinical data requirements to support proceeding to Phase 3 clinical trials with SARS-CoV-2 vaccine candidates. In addition, participants discussed concepts of trial design for these studies including trial population, endpoints and statistical considerations. For the safety evaluation in Phase 3 clinical trials (including solicited local and systemic adverse events, unsolicited adverse events, serious or other medically attended adverse events) as well as the size of the safety data base and follow-up time, the regulators recommended that they should be in the same range as required for other preventive vaccines. The protocol should include pre-specified criteria for study halt or pause, based on signals of potential vaccine-induced enhanced disease[2].

A parallel guidance from FDA in June 2020, entitled *Development and Licensure of Vaccines to Prevent COVID-19; Guidance for Industry*[3] addresses the coronavirus disease 2019 (COVID-19) public health emergency and also contains specific recommendations for evaluation of safety in clinical trials and post-licensure in addition to guidance on CMC, nonclinical data, clinical trials and diagnostic and serological assays.

[1] https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines
[2] http://www.icmra.info/drupal/news/22june2020/summary
[3] https://www.fda.gov/media/139638/download

5. Objectives of this deliverable

1. To summarize recommendations by the Brighton Collaboration and SPEAC project for vaccine safety data collection, CRF, presentation and analysis for clinical trials of CEPI funded vaccine candidates targeting COVID-19.
2. To describe the literature and consensus document on enhanced disease as a specific adverse event of special interest to COVID-19 vaccines.

2. Methods

To support and develop guidance for harmonized safety data collection with regard to COVID-19 vaccines we reviewed various source documents and used the following approaches:

1) The Brighton Collaboration template protocol for clinical trials investigating vaccines-focus on safety elements (Bonhoeffer J Vaccine. 2013 November 12; 31(47): 5602–5620. doi:10.1016/j.vaccine.2013.02.041).
2) Review of the Solidarity vaccine trial protocol that was drafted by WHO with recommendations provided to WHO. In response to a request by CEPI to provide comments to the WHO Solidarity Protocol, SPEAC outlined the safety evaluations we thought should be included for trials of COVID-19 vaccine candidates with additional input from some Meta-DSMB members. The comments are based on the WHO Solidarity Protocol version April 19, 2020[4].
   a. A sample memory aid was provided for the standardized collection of solicited local and systemic reactions
   b. BC case definitions for local and systemic reactions were reviewed and a tabulation of how local and systemic reactions following immunization may be presented was provided.
3) Evolving literature on enhanced disease following immunization was reviewed and a summary was provided.
4) A consensus meeting and subsequent discussions resulted in a published report on safety data collection for enhanced disease following immunization.

3. Results

3.1. BC template protocol for vaccine trials

The safety section in the detailed template protocol for vaccine trials as created by the Brighton Collaboration and described in the 2013 paper by Bonhoeffer et al, may be used for the COVID-19 vaccine trial protocol development (doi:10.1016/j.vaccine.2013.02.041). For broken links in the document the BC coordinator can be contacted. SPEAC recognizes the guidance is very comprehensive and recommends at least safety data collection as described below in section 3.2.

3.2. Recommendations on safety data collection for COVID-19 vaccine trials. Solidarity Protocol comments from SPEAC submitted to WHO

Because clinical trials will be occurring in sites where COVID-19 is circulating and because decisions on performance of the vaccine candidates will be made on an accelerated time scale with sample sizes smaller, possibly considerably smaller than routine, it is therefore important to maximize the information content on

[4] https://www.who.int/blueprint/priority-diseases/keyaction/WHOCOVID-2019_SolidarityVaccineTrial_ExpandedOutline_19April_Web.pdf

safety from all trial participants by standardized data collection to allow rapid comparisons among vaccine candidates and to provide the Independent Data Monitoring Committee (IDMC) with the tools to quickly evaluate initial safety data.

SPEAC recommends that the following safety evaluations be included in the SOLIDARITY protocol as well as in any safety protocol.

1. If possible, all study sites should use a common Case Report Form (CRF) despite different vaccine trials (examples provided in safety section included in **Appendix 1**
2. All participants should be followed for local (pain, tenderness, induration/swelling, erythema) and systemic (fever, fatigue/malaise, myalgia/body ache, headache, nausea, chill, arthralgia/joint pain, shivering and lymphadenopathy) reactogenicity from Day 0-7 post each injection via a subject diary (or equivalent technological app). This is the minimum information one would use to inform new vaccinees about what they might expect in terms of common AEs. An example of a memory aid for collection of these data is attached (**Appendix 2**).
3. Grading of adverse events should be standardized to promote comparison between vaccine candidates. The FDA Guidance for Industry (September 2007): "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" offers a standardized and broadly accepted tool for this purpose[5].
4. All non-solicited Adverse Events (AEs) for 30 days post-each injection should be collected
5. All participants should be followed for Serious Adverse Events (SAEs) for the duration of the study (1 year) until resolution. (NOTE: this is longer than the recommendations made in the BC trial template because of the risk of enhanced disease)
6. All participants should be followed for new onset of a chronic disease for the duration of the study.
7. All participants should be followed for the duration of the study for Adverse Events of Special Interest (AESIs)[6] that were defined by SPEAC (D2.3-COVID) and endorsed by the WHO Global Advisory Committee for Vaccine Safety[7]. As of May 27, 2020 this list (which will evolve over time) comprised the following events: enhanced disease following immunization, multisystem inflammatory syndrome in children, acute respiratory distress syndrome, acute cardiovascular injury (microangiopathy, heart failure, stress cardiomyopathy, coronary artery disease, arrhythmia), coagulation disorder (deep vein thrombosis, pulmonary embolus, cerebrovascular stroke, limb ischemia, hemorrhagic disease), generalized convulsion, Guillain Barré Syndrome, acute kidney injury, acute liver injury, anosmia, ageusia, chilblain–like lesions, single organ cutaneous vasculitis, erythema multiforme, anaphylaxis, acute aseptic arthritis, meningoencephalitis, acute disseminated encephalomyelitis, thrombocytopenia. The literature review process used to develop the list is ongoing and new AESI may be added in which case developers will be notified.
8. All pregnancies occurring on study should be followed using a standardized pregnancy registry to collect information on the maternal and neonatal outcomes. For safety data collection in pregnancy we refer to the Brighton Collaboration case definitions delivered by the Global Alignment of Immunization Safety Assessment in Pregnancy (GAIA)[8][9] including recommendations on the collection, analysis and presentation of safety data, to provide guidance on the prioritization and classification of the data to be collected in such studies, and to facilitate their applicability in various settings, including LMICs.

[5] https://www.fda.gov/media/73679/download
[6] https://brightoncollaboration.us/priority-list-aesi-covid/
[7] https://apps.who.int/iris/bitstream/handle/10665/333136/WER9528-eng-fre.pdf?ua=1
[8] https://www.sciencedirect.com/science/article/pii/S0264410X16306156
[9] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5139802/

9. All participants should be followed for clinical signs of COVID-19 disease using a standardized case definition and suggested evaluation plan (to include laboratory confirmation, clinical severity scores that will aid in evaluation of possible enhanced disease).
10. Stopping rules and pause rules should be in place prior to study start. An example of such rules might be:
    - Further enrollment and vaccinations will be halted for IDMC review/recommendation if any of the following are reported:
        - Any death occurring within the 8 days following administration of study vaccine (Day 0-7) that was not the result of trauma or accident.
        - Laryngospasm, bronchospasm, or systemic anaphylaxis within 24 hours of administration of study product
        - Two or more subjects with generalized urticaria associated with product administration within 72 hours of administration of study product
        - Ulceration, abscess, or necrosis at the injection site associated with product administration
        - Vaccine-related SAE
        - Two or more subjects with otherwise unexplained events associated with possible hypercoagulation (e.g., myocardial infarction, deep vein thrombosis, or stroke) associated within 30 days of administration of study product.
    - The study will also be halted for IDMC review/recommendation if, during the 8 days after each vaccination (Day 0-7), severe (Grade 3) vaccine-related local, systemic or laboratory abnormalities occur as pre-specified by the IDMC in a number or percentage of vaccinees that is appropriate to the profile of the individual vaccine candidates and number of participants immunized.
11. Data should be entered electronically at the site within 24 hrs of each visit and coded by experienced data managers to allow for timely IDMC review. AEs should be coded into Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. The number and percentage of subjects experiencing each specific adverse event should be tabulated by severity and relationship to study product. A complete listing of AEs for each subject should provide details including severity, relationship to study product, onset, duration and outcome.
12. The above considerations are offered realizing the need for streamlined collection of data in the face of a critical need to evaluate COVID-19 vaccine candidates quickly. For more detailed information on the reporting, analysis and presentation of safety data, please refer to the individual publications by the Brighton Collaboration on specific adverse events

As an example of how to collect the data on AEFI, we provided a sample memory aid for collection of solicited local and systemic reactions following immunization that initially was developed by IAVI for their VSV vaccine platform. We adapted their memory aid with permission, and this is included as **Appendix 2**.

To assist developers with standardized collection of safety data for across trial comparability, a guide on presentation and analysis of these solicited AE data collected in that memory aid is included as **Appendix 3** using the Brighton case definitions as a reference where available.

3.3. Enhanced disease following immunization

A literature review that was conducted to support the building of the consensus document is attached as **Appendix 4**.

One of the challenges facing rapid vaccine development for SARS-CoV-2 is the need to adequately assure the safety of these vaccines. One such safety concern is disease enhancement syndrome that occurred in the 1960s with inactivated RSV and measles vaccines. Vaccine-mediated disease enhancement is characterized by a vaccine that results in increased disease severity if the subject is later infected by the natural virus. During early trials with inactivated RSV vaccine, the vaccine did not prevent infection, 80% of those infected required hospitalization and two children died. Lung pathology in patients showed an unexpected inflammatory response with both neutrophils and eosinophils, evidence of immune complex formation and complement activation in small airways. Scientists later learned that the vaccine caused a similar disease enhancement in animals characterized by immunopathology and a T helper cell type 2 (Th2) biased response and antibody responses with poor neutralizing activity. Since that time, the animal models have been relied upon to predict safety for new RSV vaccines that are developed. Of note, the pathogenesis of RSV disease enhancement is distinct from antibody disease enhancement (ADE) which occurs for macrophage tropic viruses, demonstrated most notably for Dengue in humans and the coronavirus feline infectious peritonitis virus in cats, and is directly caused by non-neutralizing or sub-neutralizing antibodies leading to more efficient viral uptake via Fcγ receptor binding.

Since pathology consistent with the RSV vaccine enhanced disease (and perhaps ADE) has been demonstrated for some SARS-CoV-1 vaccine candidates in animal models, there is also a concern that a similar syndrome could occur in humans immunized with SARS-CoV-2 candidate vaccines.

SPEAC has responded to this challenge by first reviewing the information about enhanced disease following immunization with prior coronavirus vaccine candidates for SARS and MERS, establishing an initial literature search to gather the available information on this topic. This literature search is included as **Appendix 4**.

Because of the importance of this topic for developers with COVID-19 vaccine candidates, the Coalition for Epidemic Preparedness Innovations (CEPI) and the Brighton Collaboration (BC) Safety Platform for Emergency vACcines (SPEAC) convened a scientific working meeting on March 12 and 13, 2020 of experts in the field of vaccine immunology and coronaviruses to consider what vaccine designs could reduce safety concerns and how animal models and immunological assessments in early clinical trials can help to assess the risk. This meeting led to consensus recommendations for developers with the input of regulators attending the meeting. The summary of this meeting has been published and is included as **Appendix 5**.

4. Conclusions and recommendations

This preliminary guidance document was created to support developers of COVID-19 vaccines to use protocols that allow for harmonized safety data collection.

It provides:

Reference to the publication of the BC template vaccine trial protocol with focus on safety that was published in 2013 plus the data collection forms for safety data collection that were part of the template protocol (downloadable with this doi:10.1016/j.vaccine.2013.02.041).

Specific recommendations on how to collect safety data from SPEAC on the WHO Solidarity protocol plus a memory aid and instructions for tabulations of data on local and systemic reactions. (See Section 3.2)

Reference to the list of COVID-19 vaccine AESI that were delivered as part of the SPEAC D2.3 deliverable (available on the Brighton Collaboration website[10] and endorsed by GACVS.

Literature review and consensus document on enhanced disease following immunization (**Appendices 4 and 5**).

More than 60 Brighton case definitions for AEFI and AESI are available in the developer's toolbox and from the Brighton Collaboration website[11].

New case definitions of relevance to COVID-19 vaccines are being developed by SPEAC, these include:

- Enhanced disease following immunization (available August 2020)
- Multisystem inflammatory syndrome in children (available December 2020),
- Acute respiratory distress syndrome (available December 2020)
- Acute cardiovascular injury (microangiopathy, heart failure, stress cardiomyopathy, coronary artery disease, arrhythmia) (available December 2020),
- Coagulation disorder (deep vein thrombosis, pulmonary embolus, cerebrovascular stroke, limb ischemia, hemorrhagic disease) (available December 2020)

Supplemental data collection forms on all COVID-19 AESI are currently under development and these will supplement or round out the materials that are provided on local and systemic reactogenicity. Notifications will be sent when they are ready for review and use.

The SPEAC team recommends that developers (CEPI or otherwise funded) use the preliminary guidance document for collection of safety data, as this will allow for better comparison across trials and vaccines. Questions on availability of tools can be directed to BC-coordinator (bc-coordinator@taskforce.org).

[10] https://brightoncollaboration.us/wp-content/uploads/2020/06/SPEAC_D2.3_V2.0_COVID-19_20200525_public.pdf
[11] https://docs.google.com/spreadsheets/d/1eQf2TXXPi4Y3U1zFSo2j0pyp73gagdJx4p-VMy_qXCk/edit#gid=1666959512

APPENDIX 1: DATA COLLECTION FORMS FOR SAFETY

This is obtained in whole from appendix B in the original Brighton Collaboration template protocol (Bonhoeffer J, Imoukhuede EB, Aldrovandi G, et al. Template protocol for clinical trials investigating vaccines--focus on safety elements. Vaccine. 2013;31(47):5602-5620. doi:10.1016/j.vaccine.2013.02.041). It is openly available for download using the doi link.

We encourage the use of the template data collection forms provided in this APPENDIX. The procedure of safety data collection can typically be classified into three stages: baseline assessment, case identification, and follow-up. To prevent duplicate data collection at different stages, the data collection forms are classified into three forms for different purposes of use:

Appendix 1-I. Baseline assessment form:
to be used for baseline information collection for each participant independent from AEFI.

Appendix 1-II. AEFI report form:
to be used when an AEFI is reported at the first time.

Appendix 1-III. Follow-up form:
to be used for all follow-up visits after the above stage.

APPENDIX 1-I. BASELINE ASSESSMENT FORM

Trial ID:_____
Participant ID:_____
Medical record ID:_____
Date:_____
Site: _____

A. Vaccinee /Control
1) participant ID: ___/___/____ (DD/MM/YYYY)
2) Sex: □M □F
3) Ethnicity or race: _____
4) Weight (kg): _____
5) Height (cm): _____
6) Infants: Gestational age (weeks/days): ____/_ ___
7) Infants: Birth weight (g)_____

B. Medical History

8) Pre-vaccination signs or symptoms on day of vaccination (e.g. cold, fever):

□Yes □No □Unknown

If YES, please describe:
_____
_____
_____

9) Underlying or concomitant disease(s): □Yes □No □Unknown

If YES, please describe (including resource of the diagnoses when available, e.g. contact information of physician or hospital):
_____
_____
_____

10) Any other significant medical history including treatment (e.g., hospitalizations, pregnancy, allergies, seizures, events similar to or related to the solicited AEFI, and the resource information of the diagnoses)

_____
_____
_____

11) Any previous exposure to either the vaccine specific infectious agent or - if vector based vaccine- the vector (e.g., previous vaccination, resident of endemic area)

_____
_____
_____

12) Any medication 3 months prior to, during, and after the AEFI including prescription and non-prescription medication (e.g., herbal or homeopathic medication) as well as medication with long half-life or long term effect (e.g., immunoglobulins, blood transfusion, immunosuppressants, oral or intravenous corticosteroids), that could affect the evaluation of an AEFI, but other than treatment given for the AEFI.

□Yes □No □Unknown

If YES, please specify including the date(s), that the medication was given:
_____
_____
_____

13) Relevant family history?
□Yes □No □Unknown
If yes, please specify:
_____
_____
_____

14) Any local disease outbreaks?

□Yes □No □Unknown

If yes, please specify:
_____
_____
_____

C. Recorder:

15) Date of this report: ___/___/___ (DD/MM/YYYY)

16) Time of this report (hh:mm; 24-hour clock) ___:____

17) First name _____ 18) Last name: _____

APPENDIX 1-II. AEFI REPORT FORM

Trial ID:_____
Participant ID:_____
Medical record ID:_____
Event identifier:_____

Participant and reporter identity is confidential. Complete the form to the best of your abilities.
Should you require more space than provided to report all relevant data, please use additional pages and refer to the respective item number.

A. Source of Information/ Reporter

1) Date of this report: ___/___/___ (DD/MM/YYYY)
2) Time of this report (hh:mm; 24-hour clock) ___:____
3) First name: _____ Middle initial: _____ Last name: _____
4) Phone [+country code (area code) phone number]: +____ (_____) _____
E-mail:_____
5) Organization: _____
6) Street:_____
7) Postcode/ ZIP: _____
8) City: _____
9) State/Province: _____
10) Country:_____

11) Primary source of information:
□ Investigator □ Other(specify)_____
12) Modality to capture event:
□ Scheduled trial follow-up visit
□ Self-presentation to health facility
□ Other: _____

B. Adverse Event* (AEFI)
* If more than one event, complete one form per event

13) Initial diagnosis: _____

14) Date of diagnosis: __ _/__ _/___(DD/MM/YYYY)

15) Was the participant seen by a physician for the present complaint?
□Yes □No □Unknown

16) Contact information of Physician: _____

17) Was the participant hospitalized for the present complaint?

□Yes □No □Unknown

IF yes, contact of hospital: _____

18) Admission date: ___/___ /____ (DD/MM/YYYY)
19) Date and time of
□ onset (first sign indicative of AEFI) ___/___/___ (DD/MM/YYYY) ___/___ (hh:mm 24-hour clock) or
□ first observation (if onset unknown) ___/___/___ (DD/MM/YYYY) ___/___ (hh:mm 24-hour clock)

20) Detailed history of present complaint (e.g., type of pain, progression of symptoms and signs after the first observation)including times and dates:

_____
_____
_____
_____

21) Findings on physical examination including times, dates, and values and units of routinely measured parameters.

_____
_____
_____
_____
_____
_____
_____
_____
_____

22) Further investigations (e.g., laboratory findings, radiographs surgical and/or pathological findings and diagnoses).
For each investigation, provide investigation name, date, findings and diagnosis, and source of information:

_____
_____
_____
_____

23) Detailed record of treatments given for the AEFI including times, dates, progress of clinical condition and treatment provider:

_____
_____
_____
_____
_____

24) Was there recurrence of the event after initial AEFI or did the participant experience any AEFI to previous doses of the same vaccine?

□Yes □No □Unknown □ N/A

If YES, describe in detail including dates of occurrence:

_____
_____
_____
_____
_____

25) Any other significant medical history including treatment (e.g., hospitalizations, pregnancy, allergies, seizures, events similar to or related to the AEFI)

_____
_____
_____
_____

26) Did the event meet any criteria for a SAE?

□ Yes □ No □Unknown

□ Hospitalized for the AEFI Admission: ___/___/___
(DD/MM/YYYY) Discharge:___/___/___ (DD/MM/YYYY)

□ Not recovered; persistent signs and symptoms:

_____

□ Disability resulting from AEFI:

_____

□ Life-threatening:

_____
_____

□ Death Date of death: ___/___/___ (DD/MM/YYYY)

Cause of death is based on autopsy □ Yes □ No □Unknown
If YES, please specify

_____
_____

□ Other medically important condition (e.g., New onset chronic disease):_____

C. Most Recent Immunization(s) prior to AEFI

27) Date ___/___/___ (DD/MM/YYYY) Time ___/___ (hh:mm 24-hour clock)

28) Location (e.g. field site, hospital, physician's office, home, other): _____

29) Please list all past routine and experimental immunizations. For each immunization, provide vaccine name and administration date

	Investigational Vaccine	Co-administered Vaccine(s)
30) Vaccine		
31) Manufacturer		
32) Lot number		
33) Lot of diluent(s)		
34) Multi- or monodose vial		
35) Expir. date (DD/MM/YYYY)		
36) Volume		
37) No. of dose in series (e.g., 1st, 2nd, 3rd)		
38) Route		
39) Anatomical site of injection		
40) Device (e.g., type of syringe including needle length and gauge, biojector, electroporation, patch or other device)		
41) Source of information (e.g., vaccination record, key interview, investigator's report etc.)		
42) Any violation of administration protocol for vaccine		

APPENDIX 1-III. AEFI FOLLOW-UP FORM

Should you require more space than provided to report all relevant data, please use additional pages and refer to the respective item number.

Trial ID:_____
Participant ID:_____
Medical record ID:_____
Event identifier:_____
Date of initial report:

A. Source of Information/ Reporter

1) Date of this report: ___/___/___ (DD/MM/YYYY)
2) Time of this report (hh:mm; 24-hour clock) ___:____
3) First name: _____ Middle initial: _____ Last name: _____
4) Phone [+country code (area code) phone number]: +____ (_____) _____Fax: +____ (_____) _____
E-mail:_____
5) Organization: _____
6) Street: _____
7) Postcode/ ZIP: _____
8) City: _____
9) State/Province: _____
10) Country: _____
11) Primary source of information:
□Investigator □Other(specify)_____
12) Modality to capture event:
□ Scheduled trial follow-up visit □Self-presentation to health facility
□ Other: _____

B: AEFI Follow-up (only to be completed if form is specified as follow-up form in title of the form)

13) Final Diagnosis of AEFI: _____
14) Date of final diagnosis: ___/____/_____(DD/MM/YYYY)
15) Has causality assessment been done?

□ Yes □ No □ Unknown

If YES, is the AEFI causally related?

□ Related □ Not related □ Unknown

Describe in detail how the causality assessment has been done and contact information of the correspondent of the assessment:

_____
_____
_____

16) Has participant's condition returned to pre-vaccination health status?
□ Yes □ No □Unknown
□ Life-threatening:

_____
_____

If YES, indicate when pre-vaccination heath status was reached: ___/___/___(DD/MM/YYYY)

If NO, what is the current status? (e.g. therapeutic intervention, persistence of the event, sequelae, death, or description of any other outcome.

In the case of death, postmortem findings should be specified, if available.

_____
_____
_____

17) Did the event meet any criteria for a SAE?
□Yes □ No □Unknown
□ Hospitalized for the AEFI
Admission: ___/___/___ (DD/MM/YYYY)
Discharge:___/___/___ (DD/MM/YYYY)
□ Not recovered; persistent signs and symptoms:_____
□ Disability resulting from AEFI_____
□ Life-threatening:
□ Death
Date of death: ___/___/___ (DD/MM/YYYY)
Cause of death is based on autopsy
□ Yes □ No □ Unknown
If YES, please specify
□ Other medically important condition (e.g., New onset chronic disease):

_____

18) Was the participant revaccinated with the investigational vaccine(s)
□ Yes □ No □ Unknown
If YES, describe the doses and respective outcome

_____
_____

If NO, the reason was

□ Exclusion from further vaccination □ No further vaccination schedule

Appendix 2: Sample SPEAC Memory Aid For Solidarity Protocol

Local Reactions: If reaction is not present, record zero (0). If reaction is present, record severity grade as 1, 2, 3, or 4. Record measurements of redness or hardening in centimeters (cm).		
Group Number: ¦___¦ ☐ L arm ☐ R arm		

DAY:	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Date	¦__¦__¦¦__¦__¦__¦ (ddMMMyyyy)	¦__¦__¦¦__¦__¦__¦ (ddMMMyyyy)	¦__¦__¦¦__¦__¦__¦ (ddMMMyyyy)	¦__¦__¦¦__¦__¦__¦ (ddMMMyyyy)	¦__¦__¦¦__¦__¦__¦ (ddMMMyyyy)	¦__¦__¦¦__¦__¦__¦ (ddMMMyyyy)	¦__¦__¦¦__¦__¦__¦ (ddMMMyyyy)	¦__¦__¦¦__¦__¦__¦ (ddMMMyyyy)
Oral Temp (°C)	¦__¦__¦ . ¦__¦	¦__¦__¦ . ¦__¦	¦__¦__¦ . ¦__¦	¦__¦__¦ . ¦__¦	¦__¦__¦ . ¦__¦	¦__¦__¦ . ¦__¦	¦__¦__¦ . ¦__¦	¦__¦__¦ . ¦__¦
Time	¦__¦__¦:¦__¦__¦ am/pm	¦__¦__¦:¦__¦__¦ am/pm	¦__¦__¦:¦__¦__¦ am/pm	¦__¦__¦:¦__¦__¦ am/pm	¦__¦__¦:¦__¦__¦ am/pm	¦__¦__¦:¦__¦__¦ am/pm	¦__¦__¦:¦__¦__¦ am/pm	¦__¦__¦:¦__¦__¦ am/pm

DAY:		DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Local Reactions: If reaction not present, record zero (0). If reaction present, record severity grade as 1, 2, 3 or 4 (See below).									
Pain (pain without touching)		Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦
Tenderness (pain when area is touched)		Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦	Injection site: ¦__¦

DAY:		DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Swelling/In-duration (hardening or thickening of skin)		Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm
Redness or discoloration		Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm	Width ¦__¦__¦ . ¦__¦ cm Height ¦__¦__¦ . ¦__¦ cm

DAY:		DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Systemic Reactions: If reaction not present, record zero (0). If reaction present, record severity grade as 1, 2, 3 or 4 (See below).									
Chills		¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦
Headache		¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦
Nausea (feel like vomiting)		¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦
Malaise (feeling unwell)		¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦

DAY:		DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Myalgia (muscle pain)		¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦
Arthralgia (joint pain)		¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦	¦__¦

Severity Grading Scale for Local and Systemic Reactions

GRADE	MEANING
0	None: No signs or symptoms present.
1	Mild: Signs or symptoms causing no or little disruption of your usual daily activities
2	Moderate: Signs or symptoms causing some disruption of your usual daily activities
3	Severe: Signs or symptoms that make you unable to perform your usual daily activities
4	Very severe: Signs or symptoms that prevent you from being able to take care of yourself OR require medical intervention to prevent persistent disability or death

Important: In the event of any other observations of concern, contact the study staff at [phone number] during regular work hours. After hours, the emergency mobile is [phone number].

GENERAL INSTRUCTIONS:

This Memory Aid is given to you to record any signs or symptoms you may have following study injection. Please record the highest severity for each day and bring this card with you every time you visit the clinic.

HOW TO COMPLETE THE MEMORY AID

- Complete the Memory Aid every day, starting the day of injection (Day 0) after leaving the clinic and continue for 6 more days (for a total of 7 days). Volunteers who require help completing the Memory Aid should telephone [study site name] for assistance.
- Assess signs or symptoms, including your temperature, and fill out the Memory Aid in the evening before going to bed, around the same time every day. Document the time you assessed your symptoms in the space above.
- If you feel feverish, you may take your temperature again and record above.
- Fill out all the spaces each time. If you have no reactions, write 0 in all the boxes.

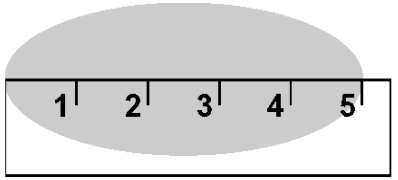
How to Take Measurements

If you notice redness of skin or skin discoloration, swelling or hardening or thickening in the area where you got your injection over the next 7 days (starting the day of injection, Day 0, after leaving the clinic and continuing for 6 more days), please measure the area using the tool the clinical staff gave you.

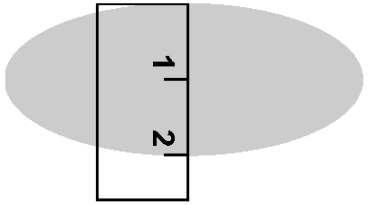
If needed, you may get someone to help you. Record the measurement in the space provided. To measure the area, do the following:

- Measure the widest part of the area from left to right
- Measure the longest part of the area from top to bottom
- Record measurements of skin discoloration or hardening in centimeters (cm)

Left to right



Top to bottom



In the example to the left, the area measures 5 centimeters (cm) from left to right and 2 centimeters (cm) from top to bottom. Record both measurements on the Memory Aid.

Contact the Research Nurse or Doctor…

- If you have any measured temperature greater than or equal to 38.6 °C.
- If you have any severe (Grade 3) or very severe (Grade 4) signs or symptoms. This includes malaise (feeling unwell), fatigue (feeling tired), muscle aches, headache, pain at the injection site, or any other event that prevents any of your daily activity or requires you to see a doctor and medical care.

If you have any concerns or questions about completing the Memory Aid or about any unusual or severe signs or symptoms you are experiencing, please contact the study staff at [phone number]. during regular work hours. After hours, the emergency mobile is [phone number].

APPENDIX 3: SPEAC GUIDANCE ON SOLICITED LOCAL AND SYSTEMIC REACTOGENICITY

In the table below, where a Brighton case definition exists, the information presented is a summary of key aspects of the case definition including the levels of certainty and guidance on how to analyze and present the relevant safety data. The specific citations for each of these case definitions are provided below the table. All are provided in the Developer's toolbox, at the Brightoncollaboration.US website and are downloadable using the respective DOIs.

Event	Brighton Case Definition exists	Key elements of CD or recommended alternate source	Data Collection Guidelines	Data Analysis Guidelines
Local Reactogenicity (all defined as present at or near the injection site)				
Local Reaction (general)	Yes	Any description of morphological or physiological change at or near the injection site. 2 levels of certainty: Level 1: assessed by a health care provider Level 2: assessed by any other person	See individual components of local reaction below for data collection. Document duration from onset to end of episode	Number of events in each category: • Level 1 or 2 of local reaction • Level 4: Reported local reaction that fails to meet level 1 or 2 • Level 5: Not a case of local reaction at the injection site For interval from immunization to new onset of local reaction, % subjects with onset from: 0-24 hrs, 25-48 hrs, 49-72 hrs, 73 hrs to 7 days, >7 days Size of reaction as % of subjects following into incremental categories of: 0-1.0 cm, then 2.5 cm increments up to <10 cm, then 5 cm increments up to <30 cm, and >30 cm.
Pain and Tenderness	Yes	For all 3 levels of certainty: an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage AND occurring at the immunization site at the time of administration or following the procedure.	Level 1 - need a subject self-report of pain or distress assessed using validated or verified instruments. For pre- or non-verbal subjects need observer report using validated age-appropriate tools. Level 2 - need other observer or reporter assessment of subject pain or distress using a validated or verified instrument. Level 3 – no additional description of pain/distress or assessment by validated method	Number of events in each category: • Level 1 -3 of pain at injection site • Level 4: Reported pain that fails to meet level 1-3 • Level 5: Not a case of pain at the injection site For interval from immunization to pain onset, % of subjects with pain onset in intervals of: <5min, 5min - ≤24 hr, 25-≤48 hr, 49-≤72hr, 73hr-≤7days, 8 - ≤14 days, >14 - ≤28 days; >28 days Validated assessment methods are provided in the published case definition. All are scored out of 10. Suggested arbitrary grading of event as: • Mild (Grade 1): score of 1-3 out of 10 • Moderate (Grade 2): score of 4-6 out of 10 • Severe (Grade 3): score of 7 or higher out of 10

Swelling	Yes	Visible enlargement of an injected limb with or without objective measurement. See 'Local reaction for levels of certainty'	Need to distinguish from injection site abscess, cellulitis, nodule and induration. Also need to distinguish swelling from induration and vice versa. Objective measurements of the size of the reaction should be made where possible, measuring greatest diameter and describing anatomical location and specify whether it includes or does not include the injection site as well as whether or not it extends from joint to joint, or crosses joints See Memory Aid	Number of events in each category: • Level 1 or 2 of swelling at injection site • Level 4: Reported swelling that fails to meet level 1 or 2 • Level 5: Not a case of swelling at the injection site The case definition suggests analyzing the % with swelling or erythema lasting: 0-24hrs; >24-48hrs; >48-72hr; >72-96hrs; >96-168hr; >7-14days; >14-21days; Where cases are limited, 2 categories are suggested (<=7 and >7 days). It is also suggested to present the % that fall into size increments for <2.5, 2.5-5, 5-<10, 10-<15, 15-<20, 20-<30, >30 cm. If relevant specify the proportion with swelling or erythema that crosses a joint, and/or extends from joint to joint
Induration	Yes	Palpable thickening, firmness or hardening of soft tissue (subcutaneous tissue, fat, fascia or muscle). See 'Local reaction for levels of certainty'		
Redness/ Erythema	No	FDA 2007 Toxicity Grading Scale defines severity: Grade 1 <15mm, Grade 2 15-30mm, Grade 3 >30mm		
Systemic Reactogenicity				
Fever	Yes	Only Level 1 of certainty: At least one elevated body temperature ≥38.0°C irrespective of device, anatomic site, age, environmental conditions.	Measure at least once/day, at same time of day, after immunization and whenever fever is suspected. Duration of surveillance depends on vaccine biologic characteristics. If fever is detected it should be followed, ideally with 2x/day(morning/evening) measurements until two consecutive measures <38°C	Number of events in each category: • Level 1 • Level 4: Reported fever that fails to meet level 1 • Level 5: Not a case of fever Duration analyzed as number of days with >1 measured temperature that meets Level 1 Degree of fever: % of subjects falling within 0.5°C increments from >38.0 °C to <41.0 °C Analyze by study arm, dose and control group as appropriate to trial design
Fatigue	Yes	Detailed case definition with 3 levels of certainty for 3 different events: a. Fatigue state	All 3 events need data to confirm it is: a new symptom; primary complaint; not relieved by rest AND interferes with normal function.	Number of events in each category: • Level 1-3 (a, b or c) • Level 4: Reported event with insufficient information to meet case definition at any level • Level 5: Not a case of fatigue.

		b. Specified fatigue syndrome c. Other fatigue syndrome Level 1 applies only to persons ≥5 years old Levels 2 & 3 apply to all ages.	Impairment should be confirmed using valid, reliable measures (see published CD). For fatigue syndromes, also need data on accompanying symptoms + signs. Exclusion criteria for all 3 events: • Concurrent onset of laboratory diagnosed medical or psychiatric disorders for which fatigue is a known symptom. • Concomitant use of a drug (therapeutic or recreational) known to cause fatigue	Duration of fatigue using following descriptors: • Acute: <1 wk in duration • Transient: ≥1 wk to <1 mo duration • Prolonged: ≥1 mo to <6 mo duration • Chronic: ≥6 mo duration Duration of each of the accompanying symptoms/signs should also be captured. Chronic fatigue state requires 4 of 8 specified symptoms to be present for >6 mo AND recommended medical, psychiatric and laboratory investigations done 6 mo or more after onset do not reveal an alternative diagnosis.
Chills	No	Not in FDA 2007 document but could use same levels of severity noted below.	Memory Aid for subjects has instructions CRF to capture memory aide data	% of subjects falling into each severity level Time to onset and duration using similar categories to those recommended for local reactions
Headache, Nausea, Malaise, Myalgia, Arthralgia	No	FDA 2007 Toxicity Grading Scale defines severity: Grades 1 (mild), 2 (moderate), 3 (severe) and 4 (potentially life-threatening).		

APPENDIX 4: CORONAVIRUS ENHANCED DISEASE LITERATURE REVIEW

Author(s): Corry Dekker

Receiver(s): Steve Black and Corry Dekker

Date: 16 MAY 2020

Diss. level: Public

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
1	Whole UV-inactivated SARS-CoV (UV-V) (Iwata Yoshikawa 2014)	Mouse BALB/c	Whole UV-inactivated SARS vaccine that includes nucleocapsid protein is reported to induce eosinophilic infiltration in mouse lungs after challenge with live SARS-CoV. TLR agonists added to UV-V vaccine, including lipopolysaccharide, poly(U), and poly(I·C) (UV-VTLR), reduced excess eosinophilic infiltration in the lungs using 6-month-old adult BALB/c mouse model. CD11bcells in the lungs of UV-V-immunized mice showed the upregulation of genes associated with the induction of eosinophils after challenge.	Vaccine-induced eosinophil immunopathology in the lungs upon SARS-CoV infection could be avoided by the TLR agonist adjuvants.	J Virol. 2014 Aug;88(15):8597-614. doi: 10.1128/JVI.00983-14. Epub 2014 May 21. Effects of Toll-Like Receptor Stimulation on Eosinophilic Infiltration in Lungs of BALB/c Mice Immunized with UV-Inactivated Severe Acute Respiratory Syndrome-Related Coronavirus Vaccine
2	SARS recombinant native full-length Spike-protein trimers (triSpike) (Jaume 2012)	In vitro	Spike envelope glycoprotein (S) is the most significant SARS-CoV neutralising and protective antigen; antibody-mediated enhancement of SARS-CoV infection with anti-SARS-CoV Spike immune-serum was observed in vitro.	Antibody-mediated infection of SARS-CoV triggers entry into human haematopoietic cells via an FcγR dependent and ACE2-, pH-, cysteine-protease-independent pathways. The antibody-mediated enhancement phenomenon is not a mandatory component of the humoral immune response elicited by SARS vaccines, as pure neutralizing antibody only could be developed. Enhanced infection of macrophages following antibody-mediated entry of feline coronavirus is responsible for the occurrence	Hong Kong Med J 2012;18 (Suppl 2):S31-6. PMID:22311359 SARS CoV subunit vaccine: antibody-mediated neutralisation and enhancement.

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
				of deadly feline infectious peritonitis.	
3	Delta inulin-based polysaccharide adjuvanted whole virus and spike protein vaccine for SARS (Honda-Okobu 2015)	Mouse BALB/c	Delta inulin induces balanced Th1 and Th2 immune responses, which contrasts with alum's marked Th2 bias. Recombinant spike protein or inactivated whole-virus vaccine candidates, alone or adjuvanted with either alum, CpG, or Advax, a new delta inulin-based polysaccharide adjuvant in adult female BALB/c mice 6 to 8 weeks of age, While all vaccines protected against lethal infection, addition of adjuvant significantly increased serum neutralizing-antibody titers and reduced lung virus titers on day 3 post challenge. Protection against eosinophilic immunopathology by vaccines containing delta inulin adjuvants correlated better with enhanced T-cell gamma interferon (IFN-) recall responses rather than reduced interleukin-4 (IL-4) responses, suggesting that immunopathology predominantly reflects an inadequate vaccine-induced Th1 response.	Whereas unadjuvanted or alum-formulated vaccines were associated with significantly increased lung eosinophilic immunopathology on day 6 post challenge, this was not seen in mice immunized with vaccines formulated with delta inulin adjuvant.	J. Virol 89:2995–3007, 2015 doi:10.1128/JVI.02980-14. Severe Acute Respiratory Syndrome-Associated Coronavirus Vaccines Formulated with Delta Inulin Adjuvants Provide Enhanced Protection while Ameliorating Lung Eosinophilic Immunopathology
4	RBD-S recombinant SARS subunit receptor-binding spike (S) protein on alum & GLA (glucopyranosyl lipid A) (Jiang 2012)	Mouse human angiotensin-converting enzyme 2 transgenic (Tg) model (proposed)	Vaccine with alum (either Alhydrogel® or aluminum phosphate), together with a synthetic Toll-like receptor 4 (TLR4) agonist known as glucopyranosyl lipid A (GLA). RBD-S polypeptide formulation, rather than the complete S protein. Outlines a 5 year plan for development of this vaccine candidate	While the recombinant S protein elicits protection, its use as a human vaccine might be limited by host immunopathology. In order to re-engineer the S protein as a safe and effective vaccine, early studies indicate that the RBD component is highly protective in laboratory animals, while significantly reducing the risk of antibody enhancement of disease [5,6,8–12]. Additional data confirm that sera from SARS convalescent patients contain robust	Expert Rev Vaccines. 2012 Dec;11(12):1405-13. doi:10.1586/erv.12.126. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome.

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
				neutralizing antibodies to RBD-S[9,12].	
5	Tc bovine human IgG immunoglobulins against MERS-CoV (Luke 2016)	No vaccine, passive Ig	Two experimental MERS-CoV vaccines were used to vaccinate two groups of transchromosomic (Tc) bovines that were genetically modified to produce large quantities of fully human polyclonal immunoglobulin G (IgG) antibodies. 2 purified Tc bovine human IgG immunoglobulins (Tc hIgG), SAB-300 (produced after Jordan strain vaccination) and SAB-301 (produced after Al-Hasa strain vaccination), also had high ELISA and neutralizing antibody titers without antibody-dependent enhancement in vitro. SAB-301 was selected for in vivo and preclinical studies. Passive immunotherapy, but antiviral effect seen when given 24 or 48h post-MERS infection.	No info	www.ScienceTranslationalMedicine.org 17 February 2016 Human polyclonal immunoglobulin G from transchromosomic bovines inhibits MERS-CoV in vivo
6	Inactivated SARS-CoV Z-1 vaccine (Luo 2018)	NHP macaques	Higher concentrations of anti-sera against SARS-CoV neutralized the SARS-CoV infection, while highly diluted anti-sera significantly increased the SARS-CoV infection and triggered ADE in macaques; effects have been reported by others. The immunogenicity and efficacy of these experimental vaccines have been evaluated in animal models such as mice, ferrets, hamsters, and non-human primates. From Wuhan Center for Virology	No groups had obvious disease symptoms, virus titers and rate of PCR+ were lower in lungs of immunized but interstitial pnx seen in vaccinated and control Rhesus macaques with no enhanced disease.	Virologica Sinica (2018) 33:201–204 https://doi.org/10.1007/s12250-018-0009-2 Evaluation of Antibody-Dependent Enhancement of SARS-CoV Infection in Rhesus Macaques Immunized with an Inactivated SARS-CoV Vaccine

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
7	Multiple animal models & vaccines for SARS-CoV, review (Roberts 2008)	Mouse Hamster Ferrets Macaques	List of vaccines as of 2008; material is dated. Several inbred mouse species (BALB/c, C57BL/6 (B6), 129S) have been shown to support SARS-CoV replication and to demonstrate pneumonitis (129S) and clinical signs of SARS disease (aged BALB/c). Golden Syrian hamster (strain LVG}, is an excellent model for SARS-CoV infection because viral replication is accompanied by pathological changes in the lungs including pneumonitis and consolidation. Ferrets support SARS-CoV replication and develop multifocal pulmonary lesions involving 5–10% of the surface area of the lung. Various strains of SARS-CoV have been evaluated in rhesus macaques, cynomolgus macaques, common marmosets, African green monkeys, squirrel monkeys and mustached tamarins (latter 2 not infected). It is unclear why the MVA-SARS-S vaccine was so poorly immunogenic (indicated by low level and transient detection of neutralizing antibodies) in ferrets since a similar vaccine was immunogenic and efficacious in mice and NHPs. It is also unclear why a memory antibody response was observed in MVA-SARS-S – immunized animals but was unable to neutralize virus or clear virus from these ferrets any faster than virus was cleared from mock-immunized ferrets.	Evidence of disease enhancement has not been seen in any of the studies where appreciable levels of neutralizing antibodies were achieved following vaccination (See references in Table 4). Furthermore, enhanced disease has not been reported in respiratory tissues or in GI tissues, which are the primary sites of viral replication in SARS-CoV infection. In a single set of experiments (Weingartl et al., 2004a), ferrets were immunized with MVA-SARS-S via IP and SC routes with 10^8 pfu of vaccine per ferret at day 0, boosted with the same regimen at day 14, and challenged intranasally at day 28. MVA-SARS-S vaccinated ferrets demonstrated low levels of neutralizing antibodies to SARS-CoV one week after the booster immunization (i.e. day 21; titer 1:40 or less), but not detectable at day 28 (<1:20), prior to challenge and no significant difference was observed in the level of virus detected in pharyngeal swabs from animals vaccinated with MVA-SARS-S and control animals following challenge	Virus Res. 2008 April ; 133(1): 20–32. doi:10.1016/j.virusres.2007.03.025. Animal Models and Vaccines for SARS-CoV Infection.

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
				with SARS-CoV. Foci of hepatic necrosis were observed in MVA-SARS-S-vaccinated animals and to a lesser extent, in animals immunized with the MVA vector alone or PBS. It is difficult to interpret the findings of hepatitis in ferrets and to determine if these findings have any relevance to the possibility of disease enhancement in SARS-vaccinated animals. Although unconfirmed, it may be possible that hepatic lesions observed in this study occur through cell-mediated mechanisms similar to that observed in the lungs in RSV infection following the use of a formalin inactivated vaccine. The finding of hepatitis following use of this MVA-vectored vaccine in ferrets merits further evaluation.	
8	b-Propiolactone Inactivated Whole Virus SARS-CoV Vaccine +/- AS01b or AS03a adjuvant (Roberts 2010)	Hamster	AS01B-adjuvanted vaccine was slightly more immunogenic than the AS03A-adjuvanted vaccine in hamster model. Although antibody titers had declined in all groups 18 wk after the second dose, the vaccinated hamsters were still partially protected from wild-type virus challenge. Vaccine with adjuvant provided better protection than non-adjuvanted WI-SARS vaccine at this later time point.	Enhanced disease was not observed in the lungs or liver of hamsters following SARS-CoV challenge, regardless of the level of serum neutralizing antibodies.	VIRAL IMMUNOLOGY Volume 23, Number 5, 2010, Pp. 509–519 DOI: 10.1089/vim.2010.0028 Immunogenicity and Protective Efficacy in Mice and Hamsters of ab-Propiolactone

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
					Inactivated Whole Virus SARS-CoV Vaccine
9	No vaccine, FIPV (Takano 2008)	Cats	Feline Infectious Peritonitis Virus model that suggests that re-infection with the same serotype induces ADE in cats infected with FIPV.	Inoculation of pathogen-free cats using FIPV serotype I enhanced the onset of FIP in cats passively immunized with FIPV serotype I-specific antibodies but not in those passively immunized with antibodies to FIPV serotype II.	J. Vet. Med. Sci. 70(12): 1315–1321, 2008 PMID:19122397 DOI: 10.1292/jvms.70.1315 Antibody-Dependent Enhancement Occurs Upon Re-Infection with the Identical Serotype Virus in Feline Infectious Peritonitis Virus Infection
10	SARS VLP vaccine, 2 whole virus vaccines and an rDNA-produced S protein +/- alum (Tseng 2012)	Mouse (BALB/c, C57BL/6) Hamsters NHP	All 4 vaccines induced serum neutralizing antibody with increasing dosages and/or alum significantly increasing responses in BALB/c and C57BL/6 mice. Significant reductions of SARS-CoV two days after challenge were seen for all vaccines and prior live SARS-CoV. All mice exhibited histopathologic changes in lungs two days after challenge including all animals vaccinated (Balb/C and C57BL/6) or given live virus, influenza vaccine, or PBS suggesting infection occurred in all. All vaccines containing S protein induced protection against infection while studies with VEE and vaccinia vector containing the N protein gene only did not. Table 2. Summary of Reported Protection and Immunopathology in Animal Model Studies with SARS Coronavirus Vaccines. Animal Model Vaccine[1] Protection[2] Immunopathology[3]	Reviews prior occurrence in the initial preclinical trial of an immunopathogenic-type lung disease among ferrets and Cynomolgus monkeys given a whole virus vaccine adjuvanted with alum and challenged with infectious SARS-CoV. Th2-type immunopathology was seen after challenge of all vaccinated animals when evaluation for immunopathology was reported except the study in hamsters with a GSK whole virus vaccine. Histopathology seen in animals given one of the SARS-CoV	PLoS One. 2012;7(4):e35421. doi: 10.1371/journal.pone.0035421. Epub 2012 Apr 20. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus.

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			Mice — Whole virus[tr] — w alum: Yes / Yes; Whole virus[25,tr] — w alum: Yes / Yes; wo alum: Yes / Yes; VLP[17,tr] — w alum: Yes / Yes; wo alum: Yes / Yes; S Protein[tr] — w alum: Yes / Yes; wo alum: Yes / Yes; VEE Vector[15] — for N protein: No / Yes; for S protein: Yes / No; Vaccinia vector[18] — for N protein: No / Yes; for S protein: Yes / ?No. Ferrets — Whole virus[11] — w alum: Yes / Yes. Nonhuman Primate[4] — Whole virus[11] — w alum: Yes / Yes. Hamsters — Whole virus[22] — w ASO1: Yes / No. [1]Reference for each indicated; tr = this report; w = with, wo = without. [2]Protection against infection (reduced lung virus after challenge). [3]Th2-type immunopathology as indicated by cellular infiltrates with prominence of eosinophils. [4]Cynomolgus monkeys. doi:10.1371/journal.pone.0035421.t002	vaccines was uniformly a Th2-type immunopathology with prominent eosinophil infiltration, confirmed with special eosinophil stains. The pathologic changes seen in all control groups lacked the eosinophil prominence. Thus, inactivated whole virus vaccines whether inactivated with formalin or beta propiolactone and whether given with or without alum adjuvant exhibited a Th2-type immunopathologic in lungs after challenge. 2 reports attributed the immunopathology to presence of the N protein in the vaccine; however, we found the same immunopathologic reaction in animals given S protein vaccine only, although it appeared to be of lesser intensity. A Th2-type immunopathologic reaction on challenge of vaccinated animals has occurred in 3/4 animal models (not in hamsters) including 2 different inbred mouse strains with 4 different types of SARS-CoV vaccines, with and without alum adjuvant“	

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11	SARS infection (no vaccine) (Yip 2016)	In vitro	Description of enhanced disease in SARS infection. Anti-SARS-CoV spike antibodies promote infection of primary human immune cells by SARS-CoV.	The antibody-dependent enhancement (ADE) infection pathway grants SARS-CoV an opportunity to infect primary human macrophages, but it does not sustain productive viral replication in the infected cells. ADE of SARS-CoV infection does not alter proinflammatory gene expression profile of primary human macrophages. Infectivity of SARS-CoV does not rely solely on the potency of target cells to bind – via Fcγ receptor II (CD32) - infectious immune complexes, but depends on the properties of the intracellular domain of the FcγRII.	Hong Kong Med J 2016;22(Suppl 4): S25-31. PMID:27390007 Antibody-dependent enhancement of SARS coronavirus infection and its role in the pathogenesis of SARS.
12	Adjuvanted (alum or VAP) and an unadjuvanted double inactivated SARS-CoV (DIV) vaccine (Bolles 2011)	Mouse (BALB/c and BALB/cBy)	DIV vaccine performed poorly in young BALB/c and BALB/cBy mice challenged with lethal homologous or heterologous strains. In contrast, DIV vaccines (both adjuvanted and unadjuvanted) performed poorly in aged-animal models. In young and aged animals, the presence of alum in the DIV vaccine formula significantly improved the induction of SARS-CoV neutralizing antibody: from moderate to high levels in young animals and from unmeasurable to moderate levels in aged animals.	Aged mice displayed increased eosinophilic immune pathology in the lungs and were not protected against significant virus replication.	J Virol. 2011 Dec;85(23):12201-15. doi: 10.1128/JVI.06048-11. Epub 2011 Sep 21. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
					increased eosinophilic proinflammatory pulmonary response upon challenge.
13	Radiation inactivated MERS-CoV vaccine+/- alum or MF59 (Agrawal 2016)	Mouse (hCD26/DPP 4 transgenic mouse model containing the human DPP4 receptor for MERS)	Inactivated MERS-CoV vaccine appears to carry a hypersensitive-type lung pathology risk from MERS-CoV infection that is similar to that found with inactivated SARS-CoV vaccines from SARS-CoV infection. Results of studies with vector vaccines point to the nucleoprotein (N) protein as responsible for the immunopathological effects seen and indicate that the S protein might be free of the risk; however, rS protein induced the pathology. The implication of the current study is that application of an inactivated MERS-CoV vaccine for prevention of MERS in humans may carry a risk for lung immunopathology if subsequently exposed to MERS-CoV."	Lung mononuclear infiltrates occurred in all groups after virus challenge but with increased infiltrates that contained eosinophils and increases in the eosinophil promoting IL-5 and IL-13 cytokines only in the vaccine groups.	Hum Vaccin Immunother. 2016 Sep;12(9):2351-6. doi: 10.1080/21645515.2016.1177688. Epub 2016 Jun 7. Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus.
14	Formalin-inactivated whole SARS virus vaccine (Darnell 2007)	Ferrets	The vaccine provided mild immune protection to the ferrets after challenge. No evidence of enhanced disease was observed in any of the ferrets.	No evidence of enhanced liver or lung disease induced by the inactivated whole-virus vaccine.	J Infect Dis. 2007 Nov 1;196(9):1329-38. Epub 2007 Sep 27. PMID:17922397 DOI: 10.1086/522431 Severe acute respiratory syndrome coronavirus infection in vaccinated ferrets.
15	Venezuelan equine encephalitis virus replicon particles (VRP)	Mouse (female BALB/c	VRP-S, but not VRP-N vaccines provide complete short- and long-term protection against homologous strain challenge in young and senescent mice.	VRP-N vaccines not only failed to protect from homologous or heterologous challenge, but	PLoS Med. 2006 Dec;3(12):e525. PMID:17194199 PMCID: PMC1716185

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	Recombinant SARS-CoV With Epidemic and Zoonotic Spike Variants (Deming 2006)	young and old)	To test VRP vaccine efficacy against a heterologous SARS-CoV, used phylogenetic analyses, synthetic biology, and reverse genetics to construct a chimeric virus (icGDO3-S) encoding a synthetic S glycoprotein gene of the most genetically divergent human strain, GDO3, which clusters among the zoonotic SARS-CoV. icGD03-S replicated efficiently in human airway epithelial cells and in the lungs of young and senescent mice, and was highly resistant to neutralization with antisera directed against the Urbani strain. Although VRP-S vaccines provided complete short-term protection against heterologous icGD03-S challenge in young mice, only limited protection was seen in vaccinated senescent animals. VRP-S vaccines provided complete short-term protection against heterologous icGD03-S challenge in young mice, only limited protection was seen in vaccinated senescent animals.	resulted in enhanced immunopathology with eosinophilic infiltrates within the lungs of SARS-CoV–challenged mice. VRP-N–induced pathology presented at day 4, peaked around day 7, and persisted through day 14, and was likely mediated by cellular immune responses	DOI: 10.1371/journal.pmed.0030525 Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants.
16	Adeno-Associated Virus Encoding Receptor-Binding Domain SARS Coronavirus Spike Protein (RBD-rAAV Vaccine) (Du 2008)	Mouse (BALB/c)	In this study, systemic, mucosal, and cellular immune responses and long-term protective immunity induced by RBD-rAAV were further characterized in a BALB/c mouse model, with comparison of the i.m. and intranasal (i.n.) routes of administration. With intranasal vaccination, higher titers of mucosal IgA and serum-neutralizing Ab were associated with lower viral load and less pulmonary pathological damage upon challenge.	No Ab-mediated disease enhancement effect was observed.	J Immunol. 2008 Jan 15;180(2):948-56. PMID:18178835 PMCID: PMC2603051 DOI: 10.4049/jimmunol.180.2.948 Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and

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					provides long-term protection against SARS-CoV infection.
17	Nucleocapsid SARS vaccine (Yasui 2008)	Mouse (BALB/c)	SARS-CoV-infected BALB/c mice that were immunized intradermally with recombinant vaccinia virus (VV) that expressed either the SARS-CoV spike (S) protein (LC16m8rVV-S) or simultaneously all the structural proteins, including the nucleocapsid (N), membrane (M), envelope (E), and S proteins (LC16m8rVV-NMES) 7-8 wk before intranasal SARS-CoV infection.	LC16mOrVV-N-immunized mice upon infection exhibited significant up-regulation of both Th1 (IFN-gamma, IL-2) and Th2 (IL-4, IL-5) cytokines and down-regulation of anti-inflammatory cytokines (IL-10, TGF-beta), resulting in robust infiltration of neutrophils, eosinophils, and lymphocytes into the lung, as well as thickening of the alveolar epithelium. Immunization with SARS nucleocapsid antigen causes severe enhanced disease on challenge.	(2008) J Immunol 181: 6337–6348. PMID:18941225 DOI: 10.4049/jimmunol.181.9.6337 Yasui F, Kai C, Kitabatake M, Inoue S, Yoneda M, et al. Prior immunization with severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV.
18	Recombinant native full-length S-protein trimer (triSpike) (Kam 2007)	Hamsters	SARS-CoV-specific serum and mucosal immunoglobulins were readily detected in immunized animals. Serum IgG blocked binding of the S-protein to the ACE2 receptor and neutralized SARS-CoV infection in vitro. Entry into human B cell lines occurred in a FcRII-dependent and ACE2-independent fashion indicating that ADE of virus entry is a novel cell entry mechanism of SARS-CoV.	Vaccinated hamsters showed no signs of enhanced lung pathology or hepatitis and viral load was undetectable or greatly reduced in lungs following challenge with SARS-CoV.	Vaccine 25 (2007) 729–740. PMID:17049691, DOI: 10.1016/j.vaccine.2006.08.011 Antibodies against trimeric S

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			Results indicate that a recombinant trimeric S protein was able to elicit an efficacious protective immune response in vivo and warrant concern in the safety evaluation of a human vaccine against SARS-CoV.	Recombinant trimeric S protein was able to elicit a protective immune response in vivo but if the observation of enhanced entry into B cells in vitro can be shown to have an in vivo correlate, this would warrant concern in the safety evaluation of a human vaccine against SARS-CoV.	glycoprotein protect hamsters against SARS-CoV challenge despite their capacity to mediate FcRII-dependent entry into B cells in vitro
19	No vaccine (Luo 2007)	NHP (rhesus macaques)	Animal model described in rhesus macaques that correlates with human SARS.	NA	Acta virologica 51: 171 – 177, 2007 PMID:1807630 Intratracheal inoculation of severe acute respiratory syndrome coronavirus in monkeys macaca rhesus
20	b-propiolactone inactivated alum-containing purified vaccine, purified vaccine + unpurified vaccine for SARS (Qin 2006)	NHP (Cynomolgus macaques)	Purified inactivated SARS vaccine could induce high levels of neutralizing antibody, protect monkeys after a SARS-CoV challenge, and be administered safely in monkeys.	Under low levels of neutralizing antibody, no exacerbation of clinical symptoms was observed when the immunized monkeys were challenged with SARS-CoV	Vaccine 24 (2006) 1028–1034. PMID: 16388880, DOI: 10.1016/j.vaccine.2005.06.038 Immunogenicity and protective efficacy in monkeys of purified inactivated Vero-cell SARS vaccine
21	In vitro studies of ADE (no vaccine) (Wang 2014)	In vitro	Observations of SARS-CoV using ADE to enhance the infectivity of a HL-CZ human promonocyte cell line.	Data suggest that antibodies against SARS-CoV spike proteins may trigger ADE effects. The data raise new	Biochem and Biophys Research Commun 451 (2014) 208–214

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			SARS-CoV is capable of replication in HL-CZ cells, and of displaying virus-induced cytopathic effects and increased levels of TNF-a, IL-4 and IL-6 two days post-infection. HL-CZ cells also expressed angiotensin converting enzyme 2 (ACE2, a SARS-CoV receptor) and higher levels of the FcgRII receptor. Higher concentrations of anti-sera against SARS-CoV neutralized SARS-CoV infection, while highly diluted anti-sera significantly increased SARS-CoV infection and induced higher levels of apoptosis. SARS-CoV ADE is primarily mediated by diluted antibodies against envelope spike proteins rather than nucleocapsid proteins. MoAbs against SARS-CoV spike proteins promoted SARS-CoV infection.	questions regarding a potential SARS-CoV vaccine, while shedding light on mechanisms involved in SARS pathogenesis.	PMID:25073113 DOI: 10.1016/j.bbrc.2014.07.090 Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins.
22	Different forms of SARS coronavirus (SARS-CoV) spike protein-based vaccines (Woo 2005)	Mouse	Prime//boost DNA with spike polypeptide boost regimen. No statistical significant difference was observed among the Th1/Th2 index among these six groups of mice with high IgG levels. Among all the combinations of vaccines examined, mice primed with SARS-CoV human codon usage optimized spike polypeptide DNA vaccines and boosted with S-peptide produced by E. coli generated the highest titer of neutralizing antibody against SARS-CoV. Authors suggest useful for civet cat vaccine?	No info	Vaccine 23 (2005) 4959–4968. PMID: 15993989, DOI: 10.1016/j.vaccine.2005.05.023 SARS coronavirus spike polypeptide DNA vaccine priming with recombinant spike polypeptide from Escherichia coli as booster induces high titer of neutralizing antibody against SARS coronavirus.
23	In vitro studies re: SARS, no vaccine (Yip 2014)	In vitro	Used primary human immune cells to evaluate their susceptibility to infection by SARS-CoV in the presence of anti-Spike antibodies. Results demonstrate that, in presence of vaccine-elicited antiviral antibodies, SARS-CoV displays an altered tropism toward primary human immune cells, which do not express the conventional virus receptor and are otherwise refractory to the virus.	Anti-Spike immune serum increased infection of human monocyte-derived macrophages by replication-competent SARS-CoV as well	Virology Journal 2014,11:82 PMID: 24885320, PMCID: PMC4018502, DOI: 10.1186/1743-422X-11-82

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				as Spike-pseudotyped lentiviral particles (SARS-CoVpp). Macrophages infected with SARS-CoV did not support productive replication of the virus. Purified anti-viral IgGs were sufficient to enhance infection. Antibody-mediated infection was dependent on signaling-competent members of the human FcγRII family, which were shown to confer susceptibility to otherwise naïve ST486 cells. Only FcγRII with intact cytoplasmic signaling domains were competent to sustain ADE of SARS-CoVpp infection.	Antibody-dependent infection of human macrophages by severe acute respiratory syndrome coronavirus.
24	Formaldehyde-inactivated Vero cell vaccine for SARS (Zhou 2005)	NHP (rhesus monkeys)	The control animals who received PBS developed atypical SARS-CoV infection after viral challenge, according to clinical, virological and pathological findings. No systematic side effects in vaccinated animals post-immunization, even in at the high dose of 5000 mcg. The 50 mcg dosage of vaccine elicited SARS-CoV specific immune responses against viral infection as compared to the partial immunity elicited by 0.5 and 5 mcg doses. This inactivated vaccine can induce effective concomitant humoral and mucosal immunity against SARS-CoV infection, is safe in monkeys.	No clinical signs or CXR changes in immunized monkeys but PBS group had mild disease. No real ADE data.	Vaccine 23 (2005) 3202–3209, PMID: 15837221, DOI: 10.1016/j.vaccine.2004.11.075 Immunogenicity, safety, and protective efficacy of an inactivated SARS-associated coronavirus vaccine in rhesus monkeys
25	Multiple SARS vaccines, review	Ferrets, Mouse,	Comprehensive review of state of the art for SARS vaccines as of 2008	Administration into ferrets of MVA-based SARS-CoV S vaccine resulted in enhanced	Virus Res. 2008 April; 133(1): 45–62.

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	(Enjuanes 2008)	Hamsters, NHP		hepatitis on challenge which has not been reported in other studies with SARS-CoV in ferrets and was not reproduced by other groups. Antibodies directed against SARS-CoV were protective and did not enhance viral infectivity in the mouse or hamster models using inactivated SARS-CoV or immunization with recombinant adenovirus vectors expressing the S and N proteins of SARS-CoV. Side effects were not observed in other animal models that were challenged with SARS-CoV and no evidence of enhanced disease upon re-challenge was shown. Cynomolgus macaques immunized with different amounts of purified virus, +/- adjuvant, showed no side effects even in the presence of low titer neutralizing antibodies. No abnormalities were observed in major organs. Immunization of mice using either S protein or whole inactivated virus or of monkeys with whole inactivated SARS-CoV most frequently resulted in the absence of side effects	doi:10.1016/j.virusres.2007.01.021. Vaccines to prevent severe acute respiratory syndrome coronavirus-induced disease.

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				after providing different types of SARS-CoV vaccines. In contrast, VEE virus expressing N protein failed to induce protection in either young or senescent animals and resulted in enhanced immunopathology following viral challenge. Co-expression of N protein in vaccine regimens which failed to simultaneously induce a strong neutralizing anti-S antibody response led to an increased number of lymphocytic and eosinophilic inflammatory infiltrates, which are also characteristic of the immune pathology observed with respiratory syncytial virus (RSV) infection following vaccination with formalin inactivated RSV.	
26	SARS Recombinant full-length Spike-protein trimers (Jaume 2011)	In vitro	We previously reported that a SARS-CoV vaccine candidate based on recombinant, full-length SARS-CoV Spike-protein trimers triggered infection of human B cell lines despite eliciting in vivo a neutralizing and protective immune response in rodents. Paper describes in vitro experiments re: mech of action for ADE using serum from immunized mice.	Anti-Spike immune serum, while inhibiting viral entry in a permissive cell line, potentiated infection of immune cells by SARS-CoV Spike-pseudotyped lentiviral particles, as well as replication-competent SARS coronavirus. Antibody-mediated infection was dependent on Fcg receptor II but did not use the endosomal/lysosomal pathway utilized by angiotensin I	J OF VIROLOGY, Oct. 2011, p. 10582–10597 Vol. 85, No. 20 0022-538X/11/$12.00 doi:10.1128/JVI.00671-11 Anti-Severe Acute Respiratory Syndrome Coronavirus Spike Antibodies Trigger

<table>
<tr><th>#</th><th>VACCINE(S) (1ST AUTHOR YR PUBLISHED)</th><th>ANIMAL MODEL</th><th>FINDINGS</th><th>ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?</th><th>REFERENCE</th></tr>
<tr><td></td><td></td><td></td><td></td><td>converting enzyme 2 (ACE2), the accepted receptor for SARS-CoV.
ADE of SARS-CoV utilizes a novel cell entry mechanism into immune cells.
Different SARS vaccine candidates elicit sera that differ in their capacity to induce ADE in immune cells despite their comparable potency to neutralize infection in ACE2-bearing cells.</td><td>Infection of Human Immune Cells via a pH- and Cysteine Protease-Independent FcgR Pathway.</td></tr>
<tr><td>27</td><td>No vaccines, review of CoV immunopathogenesis

(Perlman 2005)</td><td></td><td>
<table>
<tr><th>Mechanism</th><th>Description</th><th>Coronavirus example</th><th>References</th></tr>
<tr><td>Inflammatory storm</td><td>Excessive host response to pathogen occurs, resulting in either increased severity of localized disease or systemic disease; excessive response might be non-specific or induced by specific viral proteins; and manifestations might also occur as part of the ‘normal’ immune response required for viral clearance</td><td>MHV, FIPV, SARS-CoV</td><td>7,8,40,59</td></tr>
<tr><td>Bystander activation</td><td>T cells that are not specific for the pathogen or any host protein that is expressed at the site of inflammation are nevertheless activated (possibly by cytokines), resulting in increased tissue damage</td><td>MHV</td><td>74,111</td></tr>
<tr><td>Molecular mimicry</td><td>Pathogen and host share B- or T-cell epitopes, resulting in an autoimmune reaction in the host tissue that expresses the protein</td><td>None</td><td>–</td></tr>
<tr><td>Epitope spreading</td><td>Ongoing inflammation leads to presentation of self-epitopes, resulting in an autoimmune reaction in the host tissue that expresses the protein</td><td>MHV</td><td>69</td></tr>
<tr><td>Antibody-dependent enhancement</td><td>Antibodies specific for cell-surface glycoproteins increase virus uptake by macrophages, through cell-surface Fc receptors, resulting in disease enhancement</td><td>FIPV, possibly SARS-CoV</td><td>40,99,105</td></tr>
</table>
Fc receptor, receptor for immunoglobulin; FIPV, feline infectious peritonitis virus; MHV, murine hepatitis virus; SARS-CoV, severe- acute-respiratory-syndrome coronavirus.
</td><td>Nature Rev Immunol 5: 917–927.32. (2005)
DOI: 10.1038/nri1732

Perlman S, Dandekar AA

Immunopathogenesis of coronavirus infections: Implications for SARS.</td></tr>
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		As discussed in this Review, studies of animals that are infected with other coronaviruses indicate that excessive and sometimes dysregulated responses by macrophages and other pro-inflammatory cells might be particularly important in the pathogenesis of disease that is caused by infection with these viruses.			
28	Coronavirus-like particles (VLPs) for SARS (Lokugamage 2008)	Mouse (BALB/c)	Studied efficacy of coronavirus-like particles (VLPs) for protecting mice against severe acute respiratory syndrome coronavirus (SCoV) infection. Coexpression of SCoV S protein and E, M and N proteins of mouse hepatitis virus in 293T or CHO cells. Balb/c mice inoculated with a mixture of chimeric VLPs and alum were protected from SCoV challenge. The same groups of mice had high levels of SCoV-specific neutralizing antibodies suggesting that SCoV-specific neutralizing antibodies are important for the suppression of viral replication within the lungs.	Not seen. Despite some differences in the cellular composition of inflammatory infiltrates, no overt lung pathology in the chimeric-VLP-treated mice compared to negative control mice.	Vaccine 26: 797–808. (2008) PMID:18191004 PMCID: PMC2267761 DOI: 10.1016/j.vaccine.2007.11.092 Lokugamage KG, Yoshikawa-Iwata N, Ito N, Watts DM, Wyde PR, et al. Chimeric coronavirus-like particles carrying severe acute respiratory syndrome coronavirus (SCov) S protein protect mice against challenge with SCoV.
29	DNA vector, inactivated SARS virus, recombinant adenoviral vector encoding S with MF59 or CpG adjuvants (Kong 2005)	Mouse (BALB/c)	Cellular and humoral immune responses elicited by different combinations of gene-based and inactivated viral particles with various adjuvants have been assessed in Balb/C mice. Optimal CD8 immunity induced by DNA priming and replication-defective adenoviral vector boosting. Humoral immune response was enhanced most effectively through the use of inactivated virus with adjuvants, either MF59 or alum, and was associated with stimulation of the CD4 but not the CD8 response.	No info	J Virol 2005;79:13915–13923. [PubMed: 16254327] PMID:16254327 PMCID: PMC1280202 DOI: 10.1128/JVI.79.22.13915-13923.2005

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			Inactivated SARS virus with MF59 enhanced the CD4 and antibody response even after gene-based vaccination. The ability to boost gene-based vaccines with the adjuvanted inactivated virus shows clear enhancement of the CD4 and antibody responses. The CD8 responses are not similarly enhanced after such a boost. In contrast, DNA priming followed by rAd boosting with vectors encoding S allow induction of a strong CD8 response.		Kong WP, Xu L, Stadler K, Ulmer JB, Abrignani S, Rappuoli R, Nabel GJ. Modulation of the immune response to the severe acute respiratory syndrome spike glycoprotein by gene-based and inactivated virus immunization.
30	Spike (S) protein or inactivated whole MERS-CoV (IV) with a combined adjuvant (alum+CpG) (Deng 2018)	Mouse (transduced with Ad5-hDPP4 after final immuniza-tion)	In mice, similar levels of the anti-S protein IgG response and neutralizing activity were induced by both the S protein and IV vaccines. Immune responses against three other structural proteins, the envelope (E), membrane (M), and nucleocapsid (N) proteins, were also detected in sera of mice that received IV. No antigen-specific T-cell immunity was detected. Mice were transduced with Ad5-hDPP4 after the final immunization and were then challenged with MERS-CoV. Mice immunized with the S protein or IV showed slightly lower pathological damage in the lung, reduced antigen expression and lung virus titers. Mice that received IV formulations also showed increased protective immunity (almost no live virus was isolated from the lung).	Not seen as lung pathology scored as mod for both vaccines vs. severe for adj controls. Work differs from that of Tseng et al. in preparation of IV (inactivated with 0.4% formaldehyde vs. gamma [γ] irradiation), adjuvant formulation (Al+CpG vs. Al or MF59), and animal model (Ad5-hDPP4 transduced mice vs. hCD26/DPP4 transgenic mice).	Emerg Microbes Infect. 2018 Apr 4;7(1):60. doi: 10.1038/s41426-018-0056-7. Enhanced protection in mice induced by immunization with inactivated whole viruses compared to spike protein of middle east respiratory syndrome coronaviru s.
31	Recombinant S377–588-Fc from MERS-CoV +/- several adjuvants (Zhang 2016)	Mouse (BALB/c)	Residues 377–588 of MERS-CoV spike (S) protein receptor-binding domain (RBD) is a very promising MERS subunit vaccine candidate. Compared several adjuvants, including Freund's adjuvant, aluminum, Monophosphoryl lipid A, Montanide ISA51 and MF59 with regard to their capacity to enhance the immunogenicity of this subunit S377–588-Fc vaccine.	Not seen; MERS-CoV was detected in lungs of mice immunized with S377–588-Fc + MF59 3 and 5 days, post-infection, while high titers found in mice inoculated with	Cell Mol Immunol. 2016 Mar;13(2):180-90. doi: 10.1038/cmi.2015.03 . Epub 2015 Feb 2.

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			S377–588-Fc alone induced readily detectable neutralizing antibody and T-cell responses in immunized mice. MF59 is the most potent adjuvant as judged by superior ability to induce the highest titers of IgG, IgG1 and IgG2a subtypes, and neutralizing antibodies. Addition of MF59 also increased protection against MERS-CoV infection in BALB/c mice.	PBS + MF59 adjuvant, suggesting that in the presence of MF59, MERS-CoV RBD is highly effective in protecting mice from MERS-CoV challenge. Other formulations not tested.	Identification of an ideal adjuvant for receptor-binding domain-based subunit vaccines against Middle East respiratory syndrome coronavirus.
32	Several attenuated mutants (rSARS-CoV-MA15-E*) (Regla-Nava 2015)	Mouse (BALB/c)	To identify E protein regions and host responses that contribute to rSARS-CoV-MA15-ΔE attenuation, mutants (rSARS-CoV-MA15-E*) containing point mutations or deletions in the amino-terminal or the carboxy-terminal regions of the envelope (E) protein were generated. Small deletions and modifications within the E protein led to virus attenuation, manifested by minimal lung injury, limited neutrophil influx to the lungs, reduced expression of proinflammatory cytokines, increased anti-inflammatory cytokine levels, and enhanced $CD4^+$ and $CD8^+$ T cell counts in vivo, suggesting that these phenomena contribute to virus attenuation. The attenuated mutants fully protected mice from challenge. The major problems of using live attenuated vaccines are the possibility that the viruses may revert to virulence so they are introducing additional attenuating mutations into the Nsp1 protein to generate a safer vaccine candidate.	No enhancement seen clinically. Challenge with virulent SARS-CoV-MA15, was studied in BALB/c mice. All the nonimmunized mice lost weight and died by day 7 after rSARS-CoV-MA15 challenge. Vaccination with the attenuated mutant viruses completely protected mice from the lethal dose of SARS-CoV-MA15, as they showed no weight loss and all survived (no path).	J Virol. 2015 Apr;89(7):3870-87. doi: 10.1128/JVI.03566-14. Epub 2015 Jan 21. Severe acute respiratory syndrome coronaviruses with mutations in the E protein are attenuated and promising vaccine candidates.
33	In vitro studies with moAb MERS (Wan 2019)	In vitro	Investigated how a neutralizing monoclonal antibody (mAb), which targets the receptor-binding domain (RBD) of MERS coronavirus spike, mediates viral entry. Results showed that mAb binds to the virus-surface spike, allowing it to undergo conformational changes and become prone to proteolytic activation. mAb binds to cell-surface IgG Fc receptor, guiding viral entry through canonical viral-receptor-dependent pathways.	NA but couldn't access full article so based on abstract review	J Virol. 2019 Dec 11. pii: JVI.02015-19. doi: 10.1128/JVI.02015-19. [Epub ahead of print]

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			Antibody/Fc-receptor complex functionally mimics viral receptor in mediating viral entry.		Molecular mechanism for antibody-dependent enhancement of coronavirus entry
34	Editorial MERS, review (Li 2019)	Camels. Alpacas, NHP, Rabbit, Mouse	Whereas SARS-CoV recognizes angiotensin-converting enzyme 2 (ACE2) as a cellular receptor [9,10], MERS-CoV uses dipeptidyl peptidase 4 (DPP4) to enter target cells [11,12]. Dromedary camels, alpacas, and non-human primates are susceptible to MERS-CoV infection [21–23]; however, the virus does not infect small animals such as mice, hamsters, and ferrets [24–26]. MERS-CoV infects rabbits without causing symptoms; they also analyze the route of MERS-CoV transmission in rabbits [30]. Fan and colleagues report the development of an hDPP4-expressing mouse model through inserting hDPP4 gene into a constitutive and ubiquitous gene expression locus using CRISPR/Cas9 technology. This mouse model is susceptible to MERS-CoV infection [31]. Efficacy of a MERS-CoV S1 subunit vaccine aided by adjuvants; with reduced and delayed viral shedding in dromedary camels as well as the complete protection of alpacas from MERS-CoV infection [38]. This and other studies demonstrate that the protective efficacy of MERS vaccines positively correlates with neutralizing antibody titers in serum [38,39]. Veit and colleagues report that a MERS-CoV N protein-based vaccine, which is delivered through a modified Vaccinia virus, induces CD8+ T cell responses in a mouse model; they further identify a MERS-CoV N protein-specific CD8+ T cell epitope on the vaccine [40].	No info	Viruses. 2019 Jul 19;11(7). pii: E663. doi: 10.3390/v11070663 MERS Coronavirus: An Emerging Zoonotic Virus
35	Review of multiple MERS-CoV spike vaccines (Zhou 2018)	Mouse Camel NHP	Viral vector-, DNA-, nanoparticle-, virus-like particle (VLP)-, and S-trimer protein-based subunit vaccines [30,31,56–60]. Many of the reported full-length S-based vaccines have been tested in suitable animal models, and they demonstrated efficacy against MERS-CoV infection [31,53,57,61,62].	By optimizing and comparing five RBD fragments with different lengths, a RBD fragment containing residues 377–588 of MERS-CoV S protein is identified to induce the highest titer of antibody	Expert Rev Vaccines. 2018 Aug;17(8):677-686. doi: 10.1080/14760584.2018.1506702. Epub 2018 Aug 9.

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			A full-length S-based simian adenovirus vector vaccine (ChAdOx1) and a DNA vaccine (GLS-5300), are scheduled for clinical trials (Phase I). Viral vector-based vaccines encoding full-length S protein of MERS-CoV among which human or simian Ad, modified Vaccinia Ankara (MVA), measles virus (MV), and vesicular stomatitis virus (VSV) can be employed as the vaccine vehicles. For example, MERS-CoV S/RBD-specific systemic, mucosal, and/or cellular immune responses, as well as neutralizing antibodies against pseudotyped and live MERS-CoV, are induced in mice after immunizing them with human Ad5-based and Ad41-based or simian adenovirus vector (ChAdOx1)-based MERS-CoV full-length S-encoding vaccines [56,61,65,66], protecting hDPP4-Tg mice against MERS-CoV infection [61]. MERS-CoV S-specific systemic and mucosal antibody responses and T cell responses, particularly neutralizing antibodies, are elicited in mice and/or camels immunized with MERS-CoV full-length S-expressing MVA vaccines, protecting Ad5/hDPP4-transduced mice and dromedary camels against MERS-CoV infection [31,62,67]. MERS-CoV S-specific immune responses and neutralizing antibodies are significantly improved by the Ad (ChAdOx1)-S vaccine priming and MVA-S vaccine boosting approach [65]. In addition, a full-length S-encoding MV vaccine induces MERS-CoV S-specific antibody and T cell responses, as well as MERS-CoV neutralizing antibodies, protecting Ad5/hDPP4-transduced mice from MERS-CoV challenge [68]. VSV-based MERS-CoV full-length S vaccine is shown to elicit MERS-CoV neutralizing antibodies and T cell responses in mice and/or NHPs [69]. Other types of MERS-CoV S-based vaccines, including those based on DNA, S-trimer protein, nanoparticle, and VLP, have shown immunogenicity and/or protective efficacy against MERS-CoV infection in mouse, camel, and NHP models [30,53,57–59]. Depending on antigen doses, injection doses, or immunization routes, MERS-CoV S/RBD-specific antibody and cellular immune responses and MERS-CoV neutralizing antibody titers induced by	responses and neutralizing antibodies in immunized mice and rabbits with the capability of protecting Ad5/hDPP4 and hDPP4-Tg mice from MERS-CoV challenge with no evidence of immunological toxicity or eosinophilic immune enhancement [32,33,75,76,81]. At present, no vaccines are reported solely based on the MERS-CoV S2 subunit, potentially because of its low immunogenicity and inability to induce strong neutralizing antibodies. MERS-CoV S1 subunit, which is much longer than the RBD, also contains some non-neutralizing immunodominant epitopes. Such immunodominant epitope in SARS-CoV S1 is shown to elicit epitope sequence-dependent enhancement of viral infection [89]. Therefore, evaluation of the safety and potential immunopathological consequences is essential for full-length S and S1-based MERS vaccines before moving them to large-scale development and beginning clinical trials.	Prospects for a MERS-CoV spike vaccine.

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			viral vectored full-length S vaccines might vary [61,62], while the titer of neutralizing antibodies elicited by other vaccine types, such as nanoparticle vaccines, could be significantly affected by adjuvants, not by antigen doses [53,60]. In addition to inducing MERS-CoV-specific immune responses and neutralizing antibodies, viral vector-based full-length MERS-CoV S vaccines generally elicit anti-vector immune responses and/or neutralizing antibodies [31,62,65,68], a phenomenon that can be easily eliminated by S-based other vaccine types, such as DNA, S-trimer protein, nanoparticle, and VLP. As a short fragment inside the S1 subunit of MERS-CoV S protein, the RBD is applied as a key target for developing MERS vaccines in the categories of VLP and subunit vaccines, and their immunogenicity and efficacy have been tested in mouse, rabbits, and NHP models Mammalian cell-expressed recombinant RBD protein containing residues 377–662 of MERS-CoV S induces robust humoral systemic and mucosal immune responses and neutralizing antibodies in immunized mice [32,78]. Studies have also shown that residues 358–588 and 367–606 of MERS-CoV RBD expressed in mammalian and insect cells, respectively, elicit RBD-specific antibody or cellular immune responses and neutralizing antibodies in mice and/or rabbits [79,80]. Immunogenicity of MERS-CoV RBD-based subunit vaccines is not significantly affected by antigen dosage, but by injection doses and vaccination intervals. MERS-CoV RBD-elicited immune responses and neutralizing antibodies are significantly improved in the presence of suitable adjuvants, such as MF59, or fusion with appropriate immunopotentiators, such as Fc tag [32]. Like SARS-CoV RBD-based vaccines that induce high titers of cross-neutralizing antibodies against divergent strains of human and animal SARS-CoV [91], MERS-CoV RBD-based vaccines also elicit broad-spectrum neutralizing antibodies and cross-protective immunity against infections of divergent MERS-CoV strains from humans, camels, and antibody escape mutants [74].	RBD does not contain non-neutralizing epitopes that may cause harmful immune responses, and RBD-based vaccines do not show immunological toxicity and immunopathological effects in the animals tested [81]. Viral vectored vaccines, such as those based on Ad, against other viruses, including HIV, may induce a rapid memory immune response against the vector, enhance virus infection, or elicit limited efficacy in immunized hosts, resulting in early halting of clinical trials [93–95]. Thus, in addition to investigating immunogenicity and protection of viral-vectored MERS-CoV S candidate vaccines against MERS-CoV infection, careful design and selection of suitable viral vectors, comprehensive investigation of the possibility of anti-vector immunity in preventing MERS-CoV-specific immune responses, as well as extensive evaluation of their safety and potential toxicity, are needed before moving such vaccines forward for trials in humans.	

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				Overall, in order to further improve immunogenicity and efficacy, S-based MERS vaccines can be used alone or combined with other types of S or non-S vaccines with complementary effects by priming-boosting vaccination approaches, or conjugating with different adjuvants, and optimizing for doses, routes, or intervals. Such vaccines should be tested in different animal models, including large animal models, such as NHPs and/or camels, to confirm immunogenicity, efficacy, toxicity, and immunopathology before processing to human clinical trials.	
36	Review of host factors MERS (Widagdo 2019)	Camel	Review of the role of DPP4 and other host factors in MERS-CoV transmission and pathogenesis—such as sialic acids, host proteases, and interferons. The dromedary camel is the only animal species that has been reported to transmit this virus to humans [13,14,15,16]. MERS-CoV infection in these animals merely causes mild upper respiratory tract infection [17,18], but seroepidemiological studies showed that this virus has been circulating in dromedary camels for decades. MERS-CoV infection is mediated by the binding of S1 protein to the β-propeller domain of DPP4 [28,29,30,31]. There are 11 critical residues within the β-propeller domain that directly interact with the S1 protein [29,30,31]. These residues are quite conserved in camelids, primates, and rabbits—species shown to be susceptible to MERS-CoV [17,31,32,33]. In contrast, ferrets, rats, and mice resist MERS-CoV infection due to differences in some critical DPP4 residues [31,34,35,36].	No info	Viruses. 2019 Mar; 11(3): 280. Published online 2019 Mar19 PMCID: PMC6466079, PMID: 30893947 doi: 10.3390/v11030280 Host Determinants of MERS-CoV Transmission and Pathogenesis

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			In humans, DPP4 is absent in the nasal epithelium but present in the lower respiratory tract epithelium, mainly in type II pneumocytes [69,70]. In contrast, DPP4 is expressed in the nasal epithelium of dromedary camels [69]. This difference in DPP4 localization between humans and dromedary camels therefore explains MERS-CoV tropism in these two species. Two risk factors, i.e., smoking and chronic obstructive pulmonary disease (COPD), have been shown to upregulate DPP4 expression in the lungs.		
37	Review of MERS vaccine candidates (Schindewolf 2019)	NHP (rhesus macaques) Mouse	Studies of recovered SARS-CoV patients show that antibodies generated against the receptor binding domain (RBD) are both long-lasting (>3 years) and neutralizing [48]. RBD proteins encoding sequences from different strains of MERS-CoV have been shown to induce cross-neutralizing antibodies against divergent human and camel MERS-CoV strains as well as monoclonal antibody (mAb) escape mutants, confirming the promise of the RBD as a valid vaccine target. Vaccines targeting the N-terminal domain of S1 (NTD) offer a novel target. pVax1™ is a proprietary, optimized plasmid vaccine vector that has been developed as a MERS-CoV vaccine by encoding a consensus MERS-CoV S glycoprotein containing codon and other proprietary optimizations, as well as an IgE leader sequence to promote expression and mRNA export [71]. Intramuscular administration of this construct with electroporation induced antibodies with cross-MERS-CoV-strain neutralization and antigen-specific, polyfunctional T cell responses in rhesus macaques (completed Ph1). A vaccine regimen consisting of intramuscular administration of MERS-CoV strain England1 full-length S encoded on pVRC8400, with electroporation, and an $AlPO_4$-adjuvanted S1 protein booster, induced NAb in rhesus macaques up to 10 weeks following booster [74]. Multiple plasmid vaccine vectors encoding either full-length S or the S1 domain induce adaptive immunity and protect against MERS-CoV challenge.	Purified and adjuvanted with AddaVax™ (MF59-like), RBD protein vaccine was protective when administered IM to transgenic mice expressing human DPP4 (hDPP4), with no evidence of immunological toxicity or eosinophilic immune enhancement. While ADE has not been demonstrated with full length S from MERS-CoV, further studies must consider this as a potential issue. pVax1™ humoral and cell-mediated immune responses correlated with minimal lung pathology and reduced lung viral loads upon MERS-CoV challenge. Full-length S encoded on pVRC8400, with electroporation, and $AlPO_4$-adjuvanted S1 protein booster resulted in lower lung pathology upon challenge with	Viruses. 2019 Jan 17;11(1). pii: E74. doi: 10.3390/v11010074. Middle East Respiratory Syndrome Vaccine Candidates: Cautious Optimism.

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			Venezuelan equine encephalitis (VEE) virus replicon particles (VRPs), an alphavirus-based platform that replaces the VEE structural genes with a foreign transgene, has been shown to induce strong humoral and cellular immune responses [80,81]. A VRP encoding MERS-CoV S elicited NAb in both young and aged mice [38]. An N protein-expressing VRP protected adenovirally hDPP4-transduced mice from MERS-CoV challenge in a CD4+ T cell- and IFN-γ-dependent manner [82]. Modified vaccinia virus Ankara (MVA) [83,84] has been developed to encode full-length MERS-CoV S, induced NAb and CD8+ T cell responses in mice [85], and also protected against MERS-CoV-induced histopathology in adenovirally hDPP4-transduced mice before challenge [86]. (Ph1 underway) Ad5-vectored full-length S and S1 vaccines have been developed [93]. These elicited antigen-specific IgG and NAb when administered intramuscularly to mice with subsequent intranasal boosting. Immunizing with Ad5-vectored S followed by boosting with S nanoparticles induced S-specific IgG, NAb, and both T_h1 and T_h2 cell-mediated responses in mice, and also protected adenovirally hDPP4-transduced mice from MERS-CoV challenge. To circumvent the seroprevalence of circulating human adenoviruses, chimpanzee adenoviruses have also been developed as viral vaccine vectors [97] and have entered clinical trials [98]. ChAdOx1-MERS-CoV S vaccine protected against lethal challenge in a transgenic hDPP4 mouse model [102] A full-length or soluble form of S encoded in measles vaccine strain MV_{vac2} induced NAb, proliferation of T cells, S-specific IFN-γ production, and cytotoxic activity [104]. The vaccine also protected against MERS-CoV challenge in adenovirally hDPP4-transduced mice that were transgenic for a measles virus receptor. An NVD vector expressing MERS-CoV S was shown to induce long-lasting (up 14 weeks post-immunization) NAb titers in camels [107]. β-propiolactone-inactivated dual rabies/MERS vaccine has been proposed which incorporates the MERS-CoV S1 domain fused to rabies virus G protein on the RABV virion [111]. This vaccine elicited	the MERS-CoV strain JordanN3. Eosinophil-related lung pathology was observed for a SARS-CoV vaccine doubly inactivated with both formalin and UV irradiation [115]. This response was particularly notable in aged mice versus young mice, and following heterologous versus homologous challenge. Similarly, immunization with a gamma-irradiated MERS-CoV vaccine adjuvanted with either alum or MF59 elicited NAb and reduced viral titer upon challenge in hDPP4-transgenic mice, but induced eosinophil-related lung pathology in vaccinated mice after challenge [116]. Formalin-inactivated MERS-CoV adjuvanted with alum and oligodeoxynucleotides containing unmethylated CpG motifs was shown to elicit levels of NAb on par with those elicited by an S glycoprotein-only vaccine [117]. Moreover, the vaccine offered better protection than S alone based on reduction of lung viral titer in adenovirally hDPP4-transduced mice after MERS-CoV challenge. Remarkably,	

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			S-specific IgG and NAb and fully protected adenovirally hDPP4-transduced mice from MERS-CoV challenge. A baculovirus VLP containing S as well as MERS-CoV envelope and matrix proteins elicited RBD-specific IgG and IFN-γ responses in rhesus macaques [113]. A subsequent baculovirus VLP vaccine was developed that focused only on a fusion of the RBD from S and the immunogenic VP2 protein of canine parvovirus. This vaccine induced RBD-specific IgG, NAb, and cell-mediated responses including IFN-γ, IL-2, and IL-4 production in mice, and also activated dendritic cells in inguinal lymph nodes [114].	eosinophil-mediated vaccine-related pathology was not observed in this animal model. It has also been shown that including Toll-like receptor agonists in a UV-inactivated SARS-CoV vaccine reduced T_h2-associated pathology in lungs after challenge [118].	
38	Review SARS to MERS (Song 2019)	Mouse Hamster Ferrets NHP	Mice could be used as a stable and reproducible animal model for the evaluation of vaccines, immune-prophylaxis, and antiviral drugs against SARS-CoV. Golden Syrian and Chinese hamsters have also been evaluated and shown to be excellent models of SARS-CoV infection, owing to their high titer of virus replication in the respiratory tract, associated with diffuse alveolar damage, interstitial pneumonitis, and pulmonary consolidation. Ferrets were found to be susceptible to SARS-CoV infection but could also transmit the virus at low levels by direct contact; need more work. At least six NHP species were tested for SARS; research into the clinical signs of disease in cynomolgus and rhesus macaques gave conflicting results. The first mouse model of MERS infection reported in 2014 involved transducing animals with recombinant adenovirus 5 encoding human DPP4 (hDPP4) molecules intranasally, and this resulted in replication of MERS-CoV in the lungs. Both hDPP4-KI mouse and the MERS mouse adapted strain provide better tools to explore the pathogenesis of MERS and potential novel treatments. NHPs, such as the rhesus macaques and common marmosets, are useful models for studying the pathogenesis of mild MERS-CoV infection and evaluating novel therapies for humans, although the degree of replication and disease severity vary.	No info	Viruses 2019, 11(1), 59; https://doi.org/10.3390/v11010059 Review From SARS to MERS, Thrusting Coronaviruses into the Spotlight

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
39	MERS vaccines review (Yong 2019)	NHP (rhesus macaques) Marmosets Camels Mouse	Overall, vaccine candidates against MERS-CoV are mainly based upon the viral spike (S) protein, due to its vital role in the viral infectivity, although several studies focused on other viral proteins such as the nucleocapsid (N) protein, envelope (E) protein, and non-structural protein 16 (NSP16) have also been reported. In general, the potential vaccine candidates can be classified into six types: viral vector-based vaccine, DNA vaccine, subunit vaccine, nanoparticle-based vaccine, inactivated-whole virus vaccine and live-attenuated vaccine, which are discussed in detail. Subunit vaccine is by far the most popular method in the development of MERS vaccine, mostly focusing on the recombinant RBD of the S protein produced in heterologous expression systems. Subunit vaccines, however, are often administered along with adjuvants to boost the immunogenicity. Although the immunogenicity of VLPs-based vaccines could be enhanced by adjuvants, the VLPs themselves can serve as adjuvants which increase the immunogenicity of displayed epitopes, particularly those of smaller ones. Viral vector-based vaccine is one of the most popular approaches in developing MERS vaccines. Two out of the three candidate vaccines which have entered the clinical phase are viral vector vaccines which utilize well-studied virus replication system to display MERS-CoV antigen, thereby inducing protective immunity against MERS-CoV. DNA vaccine production does not involve virus replication, protein expression and purification, therefore reduce the cost of production. However, administration often requires an external device such as electroporator or gene gun, which eventually increases the cost of immunization. Pathological changes induced in rhesus macaques infected by MERS-CoV were the results of the host inflammatory responses triggered by the virus instead of the direct viral cytolytic activity (Prescott et al., 2018). Common marmoset also demonstrated signs of renal damage as in human cases following MERS-CoV infection, and the viral RNA could be detected in other non-respiratory organs contrary to rhesus	Vaccine candidates against SARS-CoV were initially developed based on the full-length S protein. However, these vaccines were later demonstrated to induce non-neutralizing antibodies which did not prevent MERS-CoV infection, and the immunized animals were not protected from the viral challenge instead they experienced adverse effects like enhanced hepatitis, increased morbidity, and stronger inflammatory responses (Weingartl et al., 2004; Czub et al., 2005). Many potential vaccines against MERS-CoV were also mainly focused on the same full-length S protein, raising a safety concern on the practical application of these vaccines (Du et al., 2016b). To date, no ADE has been observed in MERS-CoV. Two approaches have been suggested to mitigate the adverse effects of ADE: 1) shielding the non-neutralizing epitopes of the S proteins by glycosylation and 2) immunofocusing that aims to direct the adaptive immune responses to target only the critical neutralizing epitope to	Front Microbiol. 2019 Aug 2;10:1781. doi: 10.3389/fmicb.2019.01781. eCollection 2019. Recent Advances in the Vaccine Development Against Middle East Respiratory Syndrome-Coronavirus

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			macaques (van Doremalen and Munster, 2015; Yeung et al., 2016). Falzarano et al. (2014) also reported that the common marmoset could serve as a partially lethal animal model. MERS-CoV tropism in dromedary camels is limited to the upper respiratory tract, and is less apparent in the lower respiratory tract, contrary to rhesus macaques (Adney et al., 2014). Mice transduced by a viral vector to express human DPP4 (hDPP4) were shown to be susceptible to MERS-CoV infection, manifested by the development of pneumonia and histopathological changes in the lungs. However, viral clearance in these infected mice was observed at day-8 post-infection, failing to recapitulate severe human diseases (Zhao et al., 2014). Later, a more established transgenic mouse model expressing hDPP4 globally was developed, and it was the first lethal animal model available to evaluate MERS-CoV vaccines. Mortality was noted in these mice within days post-infection, and virus dissemination to other organs was observed with exceptionally high titer detected in the lung and brain (Agrawal et al., 2015). Another transgenic mouse model has been introduced, in which the hDPP4 gene was inserted into the genome of C57BL/6-mouse at Rosa26 locus using the CRISPR/Cas9 technology. This mouse model, namely R26-hDPP4, when infected by MERS-CoV at low dose, developed severe lung diseases related to acute respiratory symptoms (ARDS) and central nervous system (CNS). In addition, the R26-hDPP4 is also susceptible to infection by a MERS-CoV pseudovirus, serving as an alternative to test MERS-CoV vaccines in the absence of BSL-3 facility (Fan et al., 2018).	elicit a more robust protective immunity (Du et al., 2016a; Okba et al., 2017). Use of IWV as a vaccine in MERS was reported to be associated with hypersensitivity-type lung immunopathologic reaction in the mouse model (Agrawal et al., 2015),	
40	Review of MERS MoAbs and vaccines (Xu 2019)	Mouse NHP	Among all the potential targets of MERS-CoV, the spike glycoprotein (S) has been the most well-studied due to its critical role in mediating viral entry and in inducing a protective antibody response in infected individuals. The most notable studies include the recent discoveries of monoclonal antibodies and development of candidate vaccines against the S glycoprotein.	Hashem and colleagues showed that the adenovirus-based S1 vaccine may pose potential safety concerns because it may induce pulmonary perivascular hemorrhage in a MERS-CoV challenge mouse model,	Emerging Microbes & Infections 2019, VOL. 8 https://doi.org/10.1080/22221751.2019.1624482

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			Structural characterization of MERS-CoV S protein bound with these monoclonal antibodies has provided insights into the mechanisms of humoral immune responses against MERS-CoV infection.	regardless of the its full protection upon lethal viral infection. They also showed that the pulmonary pathology can be mitigated by incorporating CD40L, an immune-modulator therefore potential molecular adjuvant, into the recombinant adenovirus-based vaccine [72].	Antibodies and vaccines against Middle East respiratory syndrome coronavirus
41	MERS vaccine and treatment review (Zhou 2019)	Mouse NHP	Plasma of convalescent MERS-CoV-infected patients is useful for treatment of MERS-CoV infection clinically, but it requires a neutralizing antibody titer (e.g., 50% plaque-reduction neutralization titer:PRNT50) ≥1:80 to obtain effective therapeutic results [51–53]. Multiple vaccines described using MERS-CoV spike (S) protein receptor-binding domain (RBD)	Compared with vaccines based on MERS-CoV full-length S protein, which have the potential to attenuate neutralizing activity or enhance immune pathology, vaccines developed from MERS-CoV S-protein RBD are safer, and they do not cause immunological toxicity or eosinophilic immune enhancement [55,99,110,124]. Moreover, RBD-based therapeutic antibodies are generally more potent than non-RBD S1-based or S2-based antibodies [58,104,111]. Hence, RBD-based vaccines and therapeutic antibodies have the potential for further development as effective tools to prevent and treat MERS-CoV infection. Despite their acknowledged advantages, there are some issues associated with RBD-	Viruses 2019,11, 60; doi:10.3390/v11010060 www.mdpi.com/journal/viruses Advances in MERS-CoV Vaccines and Therapeutics Based on the Receptor-Binding Domain

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				based interventions that need to be addressed. For example, RBD is under a high level of pressure of positive selection, and mutations occur in the RBD-DPP4 binding interface that might reduce the efficacy of these treatments [100,125,126,127]. One possible way to avoid this effect, and to delay the emergence of escape mutants is to combine RBD-targeting therapeutics with those targeting other regions of the S protein, or to combine antibodies recognizing distinct epitopes within the RBD [102,128]. Such combinatorial strategies could also dramatically reduce antibody neutralization doses, providing feasible means to combat the continual threat of MERS-CoV.	
42	Review SARS, MERS (Song 2019)	Mouse, NHP	We focus on our current understanding of the epidemiology, pathogenesis, prevention, and treatment of SARS-CoV and MERS-CoV, as well as provides details on the pivotal structure and function of the spike proteins (S proteins) on the surface of each of these viruses. For building up more suitable animal models, we compare the current animal models recapitulating pathogenesis and summarize the potential role of host receptors contributing to diverse host affinity in various species.	No info	Viruses. 2019 Jan 14;11(1). pii: E59. doi: 10.3390/v11010059. From SARS to MERS, Thrusting Coronaviruses into the Spotlight

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			We outline the research still needed to fully elucidate the pathogenic mechanism of these viruses, to construct reproducible animal models, and ultimately develop countermeasures to conquer not only SARS-CoV and MERS-CoV, but also these emerging coronaviral diseases.		
43	DNA and RNA vaccines discussed, not specific to MERS and SARS (Rauch 2018)		This review discusses viral vector and nucleic acid-based vaccines (DNA and mRNA vaccines) as new approaches that might be able to tackle these challenges to global health.	No info	Front Immunol. 2018 Sep 19;9:1963. doi: 10.3389/fimmu.2018.01963. eCollection 2018. New Vaccine Technologies to Combat Outbreak Situations
44	MERS CoV review (Shokri 2018)		In this review, we have highlighted the major mechanisms of immune evasion strategies of MERS-CoV. We have demonstrated that M, 4a, 4b proteins and Plppro of MERS-CoV inhibit the type I interferon (IFN) and nuclear factor-κB signaling pathways and therefore facilitate innate immune evasion. In addition, nonstructural protein 4a (NSP4a), NSP4b, and NSP15 inhibit double-stranded RNA sensors. Therefore, the mentioned proteins limit early induction of IFN and cause rapid apoptosis of macrophages. MERS-CoV strongly inhibits the activation of T cells with downregulation of antigen presentation. In addition, uncontrolled secretion of interferon ɣ -induced protein 10 and monocyte chemoattractantprotein-1 can suppress proliferation of human myeloid progenitor cells.	No info	J Cell Physiol. 2019 Mar;234(3):2143-2151. doi: 10.1002/jcp.27155. Epub 2018 Aug 26. Modulation of the immune response by Middle East respiratory syndrome coronavirus
45	Mouse models CoV (no vaccines) (Cockrell 2018)	Mouse	In addition to assessing etiologies of known hCoVs, mouse models have clinically predictive value as tools to appraise potential disease phenotypes associated with pre-emergent CoVs. Knowledge of CoV pathogenic potential before it crosses the species barrier into the human population provides a highly desirable preclinical platform for addressing global pathogen preparedness, an overarching directive of the WHO.	No info	Mammalian Genome (2018) 29:367–383 https://doi.org/10.1007/s00335-018-9760-9

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			Although we recognize that results obtained in robust mouse models require evaluation in non-human primates, we focus this review on the current state of hCoV mouse models, their use as tractable complex genetic organisms for untangling complex hCoV–host interactions, and as pathogenesis models for preclinical evaluation of novel therapeutic interventions. Effective mouse models for SARS-CoV and MERS-CoV should minimally be able to recapitulate fatal respiratory disease having pathology similar to that observed in humans. A number of mouse models exhibiting fatal respiratory disease were developed for SARS-CoV and MERS-CoV; however, a single impediment was realized early in model development for MERS-CoV that was not confronted for SARS-CoV. The mouse orthologue of the human receptor for MERS-CoV, dipeptidyl peptidase 4 (DPP4), did not support interaction with the MERS-CoV spike glycoprotein RBD (Cockrell et al. 2014). Therefore, unlike SARS-CoV, commercially available mice were not susceptible to MERS-CoV infection and replication. A recently developed, innovative resource for genetic mapping, called the Collaborative Cross (CC), comprises a panel of recombinant inbred mouse strains containing tractable genetic diversity that approaches the genetic diversity in the human population. Using an octo-parental breeding scheme that includes classical laboratory stains (A/J, C57BL/6J, and 129/SvImJ), mouse models for human diseases (NOD/ShiLtJ for diabetes; NZO/HlLtJ for obesity), and wild-derived mouse strains (CAST/EiJ, PWK/PhJ, and WSB/EiJ), the CC captures 90% of the genetic variation present in the three major mouse subspecies (Mus musculus musculus, Mus musculus domesticus, Mus musculus castaneus) . The CC mouse platform can be used to identify novel mouse models that recapitulate human clinical outcomes resulting from pathogenic viruses.		Modeling pathogenesis of emergent and pre-emergent human coronaviruses in mice
46	Coronaviruses, no vaccine (Chen 2020)		A brief introduction of the general features of coronaviruses and describe various diseases caused by different coronaviruses in humans and animals. This review will help understand the biology	No info	J Med Virol. 2020 Jan 22. doi: 10.1002/jmv.25681. [Epub ahead of print]

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			and potential risk of coronaviruses that exist in richness in wildlife such as bats. The genome size of CoV (~30kb) is the largest among all RNA viruses, which is almost two times larger than that of the second largest RNA viruses. Based on the phylogenetic tree of CoVs, 2019-nCov is more closely related to bat-SL-CoV ZC45 and bat-SL-CoV ZXC21 and more distantly related to SARS-CoV.		Emerging coronaviruses: genome structure, replication, and pathogenesis
47	SARS and MERS CoVs pandemic potential (deWit 2016)		This Review highlights the pandemic and epidemic potential of emerging coronaviruses and discusses our current knowledge of the biology of SARS-CoV and MERS-CoV, including their transmission, their pathogenesis and the development of medical countermeasures. Key features of these viruses are the dominance of nosocomial transmission, and pathogenesis that is driven by a combination of viral replication in the lower respiratory tract and an aberrant host immune response. Similarly to all viruses in the order Nidovirales, SARS-CoV and MERS-CoV have a unique coding strategy: two-thirds of the viral RNA is translated into two large polyproteins, and the remainder of the viral genome is transcribed into a nested set of subgenomic mRNAs. Human-to-human transmission of SARS-CoV and MERS-CoV occurs mainly through nosocomial transmission; 43.5–100% of MERS cases in individual outbreaks were linked to hospitals, and very similar observations were made for some of the SARS clusters35,36. Transmission between family members occurred in only 13–21% of MERS cases and 22–39% of SARS cases. Transmission of MERS-CoV between patients was the most common route of infection (62–79% of cases), whereas for SARS-CoV, infection of health care workers by infected patients was very frequent (33–42%)35. The predominance of nosocomial transmission is probably due to the fact that substantial virus shedding occurs only after the onset of symptoms37,38, when most patients are already seeking medical care39.	One concern of vaccination in humans is vaccine-mediated enhancement of disease, a process in which the disease following infection is more severe in vaccinated individuals than in unvaccinated individuals. Although this was observed in only a small subset of vaccine studies that were carried out for SARS-CoV and has not yet been observed in any of the published MERS-CoV vaccine studies, it is an important concern. Moreover, it is unclear who to vaccinate against MERS-CoV, as healthy individuals seem to be at little risk of severe disease. Older patients or patients with underlying disease, who have the highest risk of severe MERS, would be important target populations. However, vaccination in such patients can be problematic owing to their poor immune responses,	Nat Rev Microbiol. 2016 Aug;14(8):523-34. doi: 10.1038/nrmicro.2016.81. Epub 2016 Jun 27. Review. PMID: 27344959 SARS and MERS: recent insights into emerging coronaviruses

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			An analysis of hospital surfaces after the treatment of patients with MERS showed the ubiquitous presence of viral RNA in the environment for several days after patients no longer tested positive40. Moreover, many patients with SARS or MERS were infected through super spreaders14,35,37,41–43. The immune response is essential for the resolution of an infection, but it can also result in immunopathogenesis. One indication that immunopathogenesis may contribute to SARS was the observation that viral loads were found to be decreasing while disease severity increased39,47. It is unclear whether a similar trend applies to MERS48,49. Moreover, progression to acute respiratory distress syndrome (ARDS) is associated with the upregulation of pro-inflammatory cytokines and chemokines, particularly interleukin-1β (IL-1β), IL-8, IL-6, CXC-chemokine ligand 10 (CXCL10) and CC-chemokine ligand 2 (CCL2)50,51; increased plasma levels of these molecules have been detected in patients with SARS52–55. Retrospective longitudinal studies in patients who recovered from SARS versus those who succumbed to the disease have shown an early expression of interferon-α (IFNα), IFNγ, CXCL10, CCL2 and proteins that are encoded by IFN-stimulated genes (ISGs) in all patients, but only patients who survived then had gene expression profiles that are indicative of the development of an adaptive immune response. By contrast, patients who succumbed maintained high levels of CXCL10, CCL2 and ISG-encoded proteins, whereas spike-specific antibodies were present at low levels or were absent56, which suggests that severe disease is related to the lack of a switch from an innate immune response to an adaptive immune response. The involvement of the host immune response in the pathogenesis of SARS, and most likely also that of MERS, suggests that drugs which inhibit viral replication will need to be combined with treatments that control detrimental immune responses. SARS-CoV and MERS-CoV use several strategies to avoid the innate immune response.	as has been established for influenza virus. In addition, vaccination of people with a high risk of exposure to MERS-CoV, such as health care workers, slaughterhouse workers and camel herders, is advisable.	

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48	Review of coronaviruses No vaccine (Fehr and Perlman 2015)		Vaccine development for coronaviruses faces many challenges [107]. First, for mucosal infections, natural infection does not prevent subsequent infection, and so vaccines must either induce better immunity than the original virus or must at least lessen the disease incurred during a secondary infection. Second, the propensity of the viruses to recombine may pose a problem by rendering the vaccine useless and potentially increasing the evolution and diversity of the virus in the wild [108]. Finally, it has been shown in FIPV that vaccination with S protein leads to enhanced disease [109]. Despite this, several strategies are being developed for vaccine development to reduce the likelihood of recombination, for instance by making large deletions in the nsp1 [110] or E proteins [111], rearranging the 3′ end of the genome [112], modifying the TRS sequences [113], or using mutant viruses with abnormally high mutation rates that significantly attenuate the virus [114].	No further info	Methods Mol Biol. 2015;1282:1-23. doi: 10.1007/978-1-4939-2438-7_1. Coronaviruses: an overview of their replication and pathogenesis
49	Human MERS disease No vaccine (Drosten 2014)		We studied 26 index patients with MERS-CoV infection and their 280 household contacts. The median time from the onset of symptoms in index patients to the latest blood sampling in contact patients was 17.5 days (range, 5 to 216; mean, 34.4). Probable cases of secondary transmission were identified on the basis of reactivity in two reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays. Among the 280 household contacts of the 26 index patients, there were 12 probable cases of secondary transmission (4%; 95% confidence interval, 2 to 7). Of these cases, 7 were identified by means of RT-PCR, all in samples obtained within 14 days after the onset of symptoms in index patients, and 5 were identified by means of serologic analysis, all in samples obtained 13 days or more after symptom onset in index patients. Probable cases of secondary transmission occurred in 6 of 26 clusters (23%).	No info	N Engl J Med 2014;371:828-35. DOI: 10.1056/NEJMoa1405858 Transmission of MERS-Coronavirus in Household Contacts

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			Serologic results in contacts who were sampled 13 days or more after exposure were similar to overall study results for combined RT-PCR and serologic testing. The rate of secondary transmission among household contacts of patients with MERS-CoV infection has been approximately 5%. Our findings suggest that persons in the first few decades of life without coexisting illnesses may be able to carry low levels of MERS-CoV RNA without obvious symptoms. Asymptomatic, RT-PCR–positive health care workers were identified in a recent outbreak of MERS in Jeddah.		
50	Epi SARS, MERS , flu on surfaces No vaccine (Otter 2016)		SARS-CoV, MERS-CoV, and influenza virus can survive on surfaces for extended periods, sometimes up to months. The importance of indirect contact transmission (involving contamination of inanimate surfaces) is uncertain compared with other transmission routes, principally direct contact transmission (independent of surface contamination), droplet, and airborne routes. However, influenza virus and SARS-CoV may be shed into the environment and be transferred from environmental surfaces to hands of patients and healthcare providers. Emerging data suggest that MERS-CoV also shares these properties.	No info	J Hosp Infect. 2016 Mar;92(3):235-50. doi: 10.1016/j.jhin.2015.08.027. Epub 2015 Oct 3. Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination
51	MERS CoV No vaccine (Mackay 2015)		In humans, MERS is mostly known as a lower respiratory tract (LRT) disease involving fever, cough, breathing difficulties and pneumonia that may progress to acute respiratory distress syndrome, multiorgan failure and death in 20 % to 40 % of those infected. However, MERS-CoV has also been detected in mild and influenza-like illnesses and in those with no signs or symptoms. Older males most obviously suffer severe disease and MERS patients often have comorbidities. Compared to severe acute respiratory syndrome (SARS), another sometimes- fatal zoonotic coronavirus disease that has since disappeared, MERS progresses more rapidly to respiratory failure	No info	Virol J. 2015 Dec 22;12:222. doi: 10.1186/s12985-015-0439-5. MERS coronavirus: diagnostics, epidemiology and transmission

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			and acute kidney injury (it also has an affinity for growth in kidney cells under laboratory conditions), is more frequently reported in patients with underlying disease and is more often fatal. Most human cases of MERS have been linked to lapses in infection prevention and control (IPC) in healthcare settings, with approximately 20 % of all virus detections reported among healthcare workers (HCWs) and higher exposures in those with occupations that bring them into close contact with camels. Sero-surveys have found widespread evidence of past infection in adult camels and limited past exposure among humans.		
52	CoV pathogenesis (Menachery 2017)		In this review, we explore the host and viral dynamics that shape these CoV populations for survival, amplification, and possible emergence in novel hosts.	No info	Curr Opin Virol. 2017 Apr;23:1-7. doi: 10.1016/j.coviro.2017.01.002. Epub 2017 Mar 31. Jumping species a mechanism for coronavirus persistence and survival.
53	Adenovirus-based vaccine expressing MERS-CoV S1-CD40L fusion protein (Hashem 2019)	Mouse (hDPP4 Tg^+)	Immunization of hDPP4 Tg^+ mice with a single dose of rAd5-S1/F/CD40L elicited as robust and significant specific immunoglobulin G and neutralizing antibodies as those induced with 2 doses of rAd5-S1. After MERS-CoV challenge, both vaccines conferred complete protection against morbidity and mortality, as evidenced by significantly undetectable/reduced pulmonary viral loads compared to the control group.	However, rAd5-S1– but not rAd5-S1/F/CD40L–immunized mice exhibited marked pulmonary perivascular hemorrhage post–MERS-CoV challenge despite the observed protection. Incorporation of CD40L into rAd5-based MERS-CoV S1 vaccine targeting molecule and molecular adjuvants not only enhances immunogenicity and efficacy but also prevents inadvertent pulmonary pathology after viral challenge,	J Infect Dis. 2019 Mar 26. doi:10.1093/infdis/jiz137. PubMed PMID: 30911758 A highly immunogenic, protective and safe adenovirus-based vaccine expressing MERS-CoV S1-CD40L fusion protein in transgenic human DPP4 mouse model.

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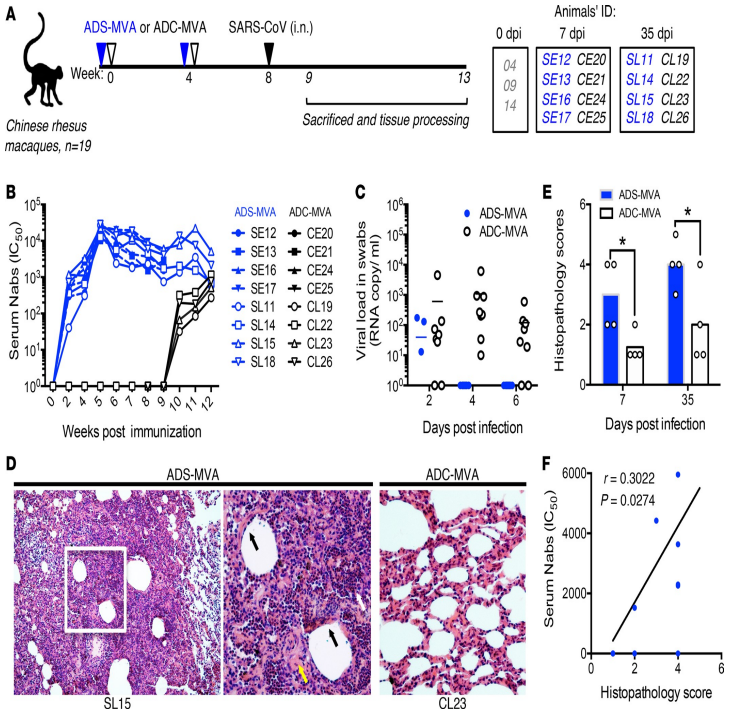
				thereby offering a promising strategy to enhance safety and potency of vaccines.	
54	MVA-SARS -CoV glycoprotein S (Liu 2019)	NHP (Chinese rhesus macaques)	An extensive set of experiments are described to elucidate mechanisms for enhanced lung pathology following administration of an MVA vaccine with SARS glycoprotein S and what happens when immune sera from immunized macaques are administered prior to live virus challenge.  	In SARS-CoV/macaque models, we determined that anti–spike IgG (S-IgG), in productively infected lungs, causes severe ALI by skewing inflammation-resolving response. Alveolar macrophages underwent functional polarization in acutely infected macaques, demonstrating simultaneously both proinflammatory and wound-healing characteristics. The presence of S-IgG prior to viral clearance, however, abrogated wound-healing responses and promoted MCP1 and IL-8 production and proinflammatory monocyte/macrophage recruitment and accumulation. Patients who eventually died of SARS displayed similarly accumulated pulmonary proinflammatory, absence of wound-healing macrophages, and faster neutralizing antibody responses. Their sera enhanced SARS-CoV–induced MCP1 and IL-8 production by human monocyte–derived wound-healing macrophages, whereas	JCI Insight. 2019; 4(4):e123158. https://doi.org/10.1172/jci.insight.123158 Anti–spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection

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				blockade of FcγR reduced such effects. Our findings reveal a mechanism responsible for virus-mediated ALI, define a pathological consequence of viral specific antibody response, and provide a potential target for treatment of SARS-CoV or other virus-mediated lung injury.	
55	SARS-CoV and SARS-CoV-2 in vitro, no vaccine (Tian 2020)	None	Here, we report for the first time that a SARS-CoV-specific human monoclonal antibody, CR3022, could bind potently with 2019-nCoV RBD (KD of 6.3 nM). The epitope of CR3022 does not overlap with the ACE2 binding site within 2019-nCoV RBD. These results suggest that CR3022 may have the potential to be developed as candidate therapeutics, alone or in combination with other neutralizing antibodies, for the prevention and treatment of 2019-nCoV infections. Interestingly, some of the most potent SARS-CoV-specific neutralizing antibodies (e.g. m396, CR3014) that target the ACE2 binding site of SARS-CoV failed to bind 2019-nCoV spike protein, implying that the difference in the RBD of SARS-CoV and 2019-nCoV has a critical impact for the cross-reactivity of neutralizing antibodies, and that it is still necessary to develop novel monoclonal antibodies that could bind specifically to 2019-nCoV RBD.	No info	Emerg Microbes Infect. 2020 Dec;9(1):382-385. doi: 10.1080/22221751.2020.1729069. Tian X, Li C, Huang A, Xia S, Lu S, Shi Z, Lu L, Jiang S, Yang Z[3] Wu Y, Ying T Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody.
56	MERS, SARS and SARS-CoV-2 pathogenesis review, no vaccine (Liu 2020)	None	Description of MERS and SARS features then the following re: SARS-CoV-2: The genetic sequence analysis revealed that the 2019-nCoV belongs to theβ-coronavirus genus, with a 79.0% nucleotide identity to SARS-CoV and 51.8% identity to MERS-CoV 34.	No info	J Med Virol. 2020 Feb 13. doi: 10.1002/jmv.25709. [Epub ahead of print]

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			Furthermore, it has been reported that nCoV-2019 is 96% identical across the entire genome to a bat coronavirus 35. Inoculation of the 2019-nCoV onto surface layers of human airway epithelial cells in vitro causes cytopathic effects and cessation of cilium beating of the cells 5. The 2019-nCoV infection was of clustering onset, is more likely to affect older males with comorbidities, and can result in severe and even fatal respiratory diseases 36,37. The major clinical symptoms resulting from 2019-nCoV infection at the prodromal phase include fever, dry cough, myalgia, fatigue, and diarrhea38. Many patients also developed dyspnea and lymphopenia. Complications of 2019-nCoV infections included acute respiratory distress syndrome, RNAaemia, acute cardiac injury, and secondary (super-)infections 38. All reported cases, including asymptomatic patients, had abnormal findings concerning the chest CT as indicated by bilateral ground glass opacity 6,38. The prototypical findings of chest CT images of seriously ill patients requiring ICU admission were bilateral multiple lobular and subsegmental areas of consolidation38. Initial plasma IL-1β, IL-1Rα, IL-7, IL-8, IL-9, IL-10, basic FGF, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1A, MIP1B, PDGF, TNF-α, and VEGF concentrations were higher in 2019-nCoV-infected patients as compared to healthy controls. Moreover, ICU patients showed higher plasma levels of IL-2, IL-7, IL-10, GSCF, IP10, MCP1, MIP1A, and TNF-α than non-ICU patients 38. These results suggest that immunopathology may also play a relevant role in the development of disease severity.		Liu J, Zheng X, Tong Q, Li W, Wang B, Sutter K, Trilling M, Lu M, Dittmer U, Yang D Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV.
57	SARS and MERS CoV review of immune response to infection, no vaccine (Li 2020)	None	In this review, we provide an update on CoV infections and relevant diseases, particularly the host defense against CoV-induced inflammation of lung tissue, as well as the role of the innate immune system in the pathogenesis and clinical treatment. By comparing T-cell-deficient BALB/c mice (transduced by ad5-hdp4) with controls and B-cell-deficient mice, some researchers	No info	J Med Virol. 2020 Jan 25. doi: 10.1002/jmv.25685. [Epub ahead of print] Li G, Fan Y Lai Y Han T, Li Z, Zhou P, Pan

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			determined that T cells could survive in the infected lungs and destroy the infected cells.75 The depletion of CD8+ T cells does not affect and delay viral replication at the time of infection with SARS-CoV.77,78 Depletion of CD4+ T cells is associated with reduced pulmonary recruitment of lymphocytes and neutralizing antibody and cytokine production, resulting in a strong immune-mediated interstitial pneumonitis and delayed clearance of SARS-CoV from lungs.79 MERS-CoV induces T cell apoptosis by activating the intrinsic and extrinsic apoptosis pathways. A novel BH3-like region located in the C-terminal cytosolic domain of SARS-CoV protein mediates its binding to Bcl-xL and induced T-cell apoptosis.83 During the later stage of infection, depletion of T cells having antiviral effects may prolong the infection and promote viral survival.84 SARS-CoV-specific T cells have been screened in SARS convalescent patients. All the detected memory T cell responses are directed at SARS-CoV structural proteins. Two CD8+T cell responses to SARS-CoV membrane (M) and Nucleocapsid (N) protein are characterized by measuring their HLA restriction and minimal T cell epitope regions. Further, these reactions are found to last up to 11 years after infection. Absence of cross-reactivity of these CD8+T cell responses against the MERS-CoV is also demonstrated.78 Reports show that humoral immunity is essential to control the persistent phase of CoV infection. SARS-CoV infection activates the complement pathway and complement signaling contributes to disease.92		P, Wang W, Hu D, Liu X, Zhang Q, Wu J. Coronavirus infections and immune responses.
58	No vaccine, EM study SARS-CoV spike (Gui, 2017)	In vitro	We report here the cryo-EM structure determination of the SARS-CoV S glycoprotein trimer in four different conformations. Structural analyses revealed that these conformations are different in the position of one C-terminal	No info	Cell Res. 2017 Jan;27(1):119-129. doi: 10.1038/cr.2016.152. Epub 2016 Dec 23.

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			domain 1 (CTD1), which functions as the RBD of the S glycoprotein trimer. Structural comparisons further indicated that a "down" to "up" positional change of the CTD1 switches the S glycoprotein trimer from receptor-binding inactive to active state, which is a prerequisite for the binding of SARS-CoV receptor ACE2 and for the neutralization by monoclonal antibodies.		Gui M, Song W, Zhou H, Xu J, Chen S, Xiang Y, Wang X. Cryo-electron microscopy structures of the SARS-CoV spike glycoprotein reveal a prerequisite conformational state for receptor binding.
59	No vaccine, in vitro studies using survivor SARS and MERS patient antibodies (Walls, 2019)		The trimeric spike transmembrane glycoprotein S mediates entry into host cells and is the major target of neutralizing antibodies. To understand the humoral immune response elicited upon natural infections with coronaviruses, we structurally characterized the SARS-CoV and MERS-CoV S glycoproteins in complex with neutralizing antibodies isolated from human survivors. Although the two antibodies studied blocked attachment to the host cell receptor, only the anti-SARS-CoV S antibody triggered fusogenic conformational changes via receptor functional mimicry. These results provide a structural framework for understanding coronavirus neutralization by human antibodies and shed light on activation of coronavirus membrane fusion, which takes place through a receptor-driven ratcheting mechanism. In summary, coronaviruses appear to have evolved a fine-tuned balance between masking of the RBM, to limit neutralization by the humoral host immune response, and their necessary exposure, to enable receptor recognition and infection of host cells.	No info	Cell. 2019 Feb 21;176(5):1026-1039.e15. doi: 10.1016/j.cell.2018.12.028. Epub 2019 Jan 31. Walls AC, Xiong X, Park YJ, Tortorici MA, Snijder J, Quispe J, Cameroni E, Gopal R, Dai M, Lanzavecchia A, Zambon M, Rey FA, Corti D, Veesler D. Unexpected Receptor Functional Mimicry Elucidates Activation of Coronavirus Fusion.

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60	No vaccines, animal model SARS-CoV-2 (Bao, 2020)	Mice, hACE2 transgenic. 6-11-month male and female WT mice (WT-HB-01) and hACE2 mice (ACE2-HB-01)	Based on angiotensin converting enzyme 2 (ACE2) as the cell entry receptor of SARS-CoV, we used the hACE2 transgenic mice infected with SARS-CoV-2 to study the pathogenicity of the virus. Weight loss and virus replication in lung were observed in hACE2 mice infected with SARS-CoV-2. The typical histopathology was interstitial pneumonia with infiltration of significant lymphocytes and monocytes in alveolar interstitium, and accumulation of macrophages in alveolar cavities. Viral antigens were observed in the bronchial epithelial cells, alveolar macrophages and alveolar epithelia. The phenomenon was not found in wild type mice with SARS-CoV-2 infection. The pathogenicity of SARS-CoV-2 in hACE2 mice was clarified and the Koch's postulates were fulfilled as well, and the mouse model may facilitate the development of therapeutics and vaccines against SARS-CoV-2. The pathogenicity of SARS-CoV-2 seems mild compared to SARS-CoV in mice, the latter caused extrapulmonary organ damage, includes brain, kidney, intestine, heart and liver, furthermore, the neurons are susceptible for SARS-CoV infection, and cerebral vasculitis and hemorrhage were observed in hACE2 transgenic mice10,11. However, only interstitial pneumonia was observed in SARS-CoV-2-infected hACE2 mice, implying the disparity in pathogenicity of the coronavirus.	No info	bioRxiv preprint doi: https://doi.org/10.1101/2020.02.07.939389. Linlin Bao, Wei Deng, Baoying Huang, et al The Pathogenicity of SARS-CoV-2 in hACE2 Transgenic Mice

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61	No vaccine, comparison sequence data (Ahmed, 2020)		By screening the experimentally determined SARS-CoV-derived B cell and T cell epitopes in the immunogenic structural proteins of SARS-CoV, we identified a set of B cell and T cell epitopes derived from the spike (S) and nucleocapsid (N) proteins that map identically to SARS-CoV-2 proteins. As no mutation has been observed in these identified epitopes among the 120 available SARS-CoV-2 sequences (as of 21 February 2020), immune targeting of these epitopes may potentially offer protection against this novel virus. For the T cell epitopes, we performed a population coverage analysis of the associated MHC alleles and proposed a set of epitopes that is estimated to provide broad coverage globally, as well as in China. Our findings provide a screened set of epitopes that can help guide experimental efforts towards the development of vaccines against SARS-CoV-2. We found that only 23% and 16% of known SARS-CoV T cell and B cell epitopes map identically to SARS-CoV-2, respectively, and with no mutation having been observed in these epitopes among the available SARS-CoV-2 sequences (as of 21 February 2020). This provides a strong indication of their potential for eliciting a robust T cell or antibody response in SARS-CoV-2.	No info	Viruses. 2020 Feb 25;12(3). pii: E254. doi: 10.3390/v12030254. Ahmed SF, Quadeer AA, McKay MR. Preliminary Identification of Potential Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological Studies.
62	No vaccine, case report COVID-19 (Lan 2020)		Four patients (HCWs) with COVID-19 who met criteria for hospital discharge or discontinuation of quarantine in China (absence of clinical symptoms and radiological abnormalities and 2 negative RT-PCR test results) had positive RT-PCR test results 5 to 13 days later. These findings suggest that at least a proportion of recovered patients still may be virus carriers. Although no family members were infected, all reported patients were medical professionals and took special care during home quarantine.	No, asymptomatic infection post-quarantine in this group although other reports describe increased symptoms can occur with postulated second infection	JAMA. 2020 Feb 27. doi: 10.1001/jama.2020.2783. [Epub ahead of print] Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, Xu H Positive RT-PCR Test Results in Patients Recovered From COVID-19.

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63	No vaccine, review SARS, MERS, COVID-19 (Li, 2020)	Mice, transgenic	SARS-CoV-2 shares highly homological sequence with SARS-CoV, and causes acute, highly lethal pneumonia (COVID-19) with clinical symptoms similar to those reported for SARS-CoV and MERS-CoV. The most characteristic symptom of COVID-19 patients is respiratory distress, and most of the patients admitted to the intensive care could not breathe spontaneously. Additionally, some COVID-19 patients also showed neurologic signs such as headache, nausea and vomiting. Increasing evidence shows that coronaviruses are not always confined to the respiratory tract and that they may also invade the central nervous system inducing neurological diseases. The infection of SARS-CoV has been reported in the brains from both patients and experimental animals, where the brainstem was heavily infected. Furthermore, some coronaviruses have been demonstrated able to spread via a synapse-connected route to the medullary cardiorespiratory center from the mechano- and chemoreceptors in the lung and lower respiratory airways. In light of the high similarity between SARS-CoV and SARS-CoV2, it is quite likely that the potential invasion of SARS-CoV2 is partially responsible for the acute respiratory failure of COVID-19 patients. Awareness of this will have important guiding significance for the prevention and treatment of the SARS-CoV-2-induced respiratory failure. Experimental studies using transgenic mice further revealed that either SARS-CoV 30 or MERS-COV 11, when given intranasally, could enter the brain, possibly via the olfactory nerves, and thereafter rapidly spread to some specific brain areas including thalamus and brainstem. It is noteworthy that in the mice infected with low inoculum doses of MERS-CoV virus particles were detected only in the brain, but not in the lung, which indicates that the infection in the CNS was more important for the high mortality observed in the infected mice 11.		J Med Virol. 2020 Feb 27. doi: 10.1002/jmv.25728. [Epub ahead of print] Li YC[1], Bai WZ[2], Hashikawa T[3]. The neuroinvasive potential of SARS-CoV2 may be at least partially responsible for the respiratory failure of COVID-19 patients.

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			Among the involved brain areas, the brainstem has been demonstrated to be heavily infected by SARS-CoV 30, 35 or MERS-CoV 11. The exact route by which SARS-CoV or MERS-COV enters the CNS is still not reported. However, hematogenous or lymphatic route seems impossible, especially in the early stage of infection, since almost no virus particle was detected in the non-neuronal cells in the infected brain areas 32-34. On the other hand, increasing evidence shows that CoVs may first invade peripheral nerve terminals, and then gain access to the CNS via a synapse-connected route 9-10, 19, 36 . The trans-synaptic transfer has been well documented for other coronaviruses, such as HEV67 9-10, 18-19 and avian bronchitis virus 36-37.		
64	No vaccine, brief review supporting use of convalescent plasma COVID-19 (Chen, 2020)		Convalescent plasma or immunoglobulins have been used as a last resort to improve the survival rate of patients with SARS whose condition continued to deteriorate despite treatment with pulsed methylprednisolone. Moreover, several studies showed a shorter hospital stay and lower mortality in patients treated with convalescent plasma than those who were not treated with convalescent plasma.2–4 In 2014, the use of convalescent plasma collected from patients who had recovered from Ebola virus disease was recommended by WHO as an empirical treatment during outbreaks.5 A protocol for the use of convalescent plasma in the treatment of Middle East respiratory syndrome coronavirus was established in 2015.6 In terms of patients with pandemic 2009 influenza A H1N1 (H1N1pdm09) virus infection, a prospective cohort study by Hung and colleagues showed a significant reduction in the relative risk of mortality (odds ratio 0·20 [95% CI 0·06–0·69], p=0·01) for patients treated with convalescent plasma.7 Additionally, in a subgroup analysis, viral load after convalescent plasma treatment was significantly lower on days 3, 5, and 7 after intensive care unit admission. No adverse events were observed.	No info	Lancet Infect Dis. 2020 Feb 27. pii: S1473-3099(20)30141-9. doi: 10.1016/S1473-3099(20)30141-9. [Epub ahead of print] Chen L, Xiong J, Bao L, Shi Y Convalescent plasma as a potential therapy for COVID-19.

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			A multicentre, prospective, double-blind, randomised controlled trial by Hung and colleagues showed that using convalescent plasma from patients who recovered from the influenza A H1N1pdm09 virus infection to treat patients with severe influenza A H1N1 infection was associated with a lower viral load and reduced mortality within 5 days of symptom onset.8 A meta-analysis by Mair-Jenkins and colleagues showed that the mortality was reduced after receiving various doses of convalescent plasma in patients with severe acute respiratory infections, with no adverse events or complications after treatment.9 Another meta-analysis by Luke and colleagues identified eight studies involving 1703 patients with 1918 influenza pneumonia from 1918 to 1925 who received an infusion of influenza-convalescent human blood products, which showed a pooled absolute reduction of 21% (95% CI 15–27; p<0.001) in the overall case fatality rate at low risk of bias.		
65	Avian infectious bronchitis virus (IBV) QX genotype (GI-19 lineage) S1 subunit replacing a Mass+793B based vaccination (Franzo, 2019)	Chickens (broiler in Italy)	Infectious bronchitis virus (IBV) is a widespread avian coronavirus, whose control relies mainly on extensive vaccine administration. Unfortunately, the continuous emergence of new vaccine-immunity escaping variants prompts the development of new vaccines. In the present work, a molecular epidemiology study was performed to evaluate the potential role of homologous vaccination in driving IBV evolution. This was undertaken by assessing IBV viral RNA sequences from the ORF encoding the S1 portion of viral surface glycoprotein (S) before and after the introduction of a new live vaccine on broiler farms in northern-Italy. The results of several biostatistics analyses consistently demonstrate the presence of a higher pressure in the post-vaccination period. Natural selection was detected essentially on sites located on the protein surface, within or nearby domains involved in viral attachment or related functions. This evidence strongly supports the action of vaccine-induced immunity in conditioning viral evolution, potentially leading to the emergence of new vaccine-escape variants.	No info	Vet Res. 2019 Nov 9;50(1):92. doi: 10.1186/s13567-019-0713-4. Franzo G, Legnardi M, Tucciarone CM, Drigo M, Martini M, Cecchinato M. Evolution of infectious bronchitis virus in the field after homologous vaccination introduction.

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			The great plasticity of rapidly-evolving RNA-viruses in response to human intervention, which extends beyond the poultry industry, is demonstrated, claiming further attention due to their relevance for animal and especially human health. IBV vaccine immunity is not sterilizing and a certain viral persistence in vaccinated animals is possible [42]. The scenario is further worsened by the typically partial coverage achieved by routine vaccination protocols in field conditions that, although usually effective in preventing clinical outbreaks and reducing the infectious pressure, facilitates the circulation of field viruses in a partially immunized population [21, 43]. A previous study based on a phylodynamic approach, performed in the same geographic area and timeframe, demonstrated the benefits of the homologous vaccination introduction in reducing viral population size and outbreak frequency [21]. Nevertheless, the IBV QX genotype has continued to circulate in Italy.		
66	SARS-CoV-2 patient samples, sequencing (Shen, 2020)	None	We have conducted metatranscriptome sequencing for the bronchoalveolar lavage fluid of eight SARS-CoV-2 patients, 25 community-acquired pneumonia (CAP) patients, and 20 healthy controls. The median number of intra-host variants was 1-4 in SARS-CoV-2 infected patients, which ranged between 0 and 51 in different samples. The distribution of variants on genes was similar to those observed in the population data (110 sequences). However, very few intra-host variants were observed in the population as polymorphism, implying either a bottleneck or purifying selection involved in the transmission of the virus, or a consequence of the limited diversity represented in the current polymorphism data. Although current evidence did not support the transmission of intra-host variants in a person-to-person spread, the risk should not be overlooked.	No info	Clin Infect Dis. 2020 Mar 4. pii: ciaa203. doi: 10.1093/cid/ciaa203. [Epub ahead of print] Shen Z, Xiao Y, Kang L, Ma W, Shi L, Zhang L, Zhou Z, Yang J, Zhong J, Yang D, Guo L, Zhang G, Li H, Xu Y, Chen M, Gao Z, Wang J, Ren L, Li M. Genomic diversity of SARS-CoV-2 in Coronavirus Disease 2019 patients

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			The microbiota in SARS-CoV-2 infected patients was similar to those in CAP, either dominated by the pathogens or with elevated levels of oral and upper respiratory commensal bacteria. SARS-CoV-2 evolves in vivo after infection, which may affect its virulence, infectivity, and transmissibility. Although how the intra-host variant spreads in the population is still elusive, it is necessary to strengthen the surveillance of the viral evolution in the population and associated clinical changes.		
67	Review of animal models and assays for SARS vaccines (Roberts 2006)	Mice, hamsters, ferrets. NHP	Available data suggest that vaccines should be based on the 180 kDa viral spike protein, S, the only significant neutralization antigen capable of inducing protective immune responses in animals. In the absence of clinical cases of SARS, candidate vaccines should be evaluated for efficacy in animal models, and although it is uncertain whether the United States Food and Drug Administration's "animal rule" would apply to licensure of a SARS vaccine, it is important to develop standardized animal models and immunological assays in preparation for this eventuality. This report summarizes the recommendations from a WHO Technical Meeting and provides guidance on the use of animal models, and outlines the steps to develop standard reagents and assays for immunological evaluation of candidate SARS vaccines. SARS-CoV-like viruses that were isolated from civets and raccoon dogs had more than 99% homology with human SARS-CoV, with major differences found in ORF8, whose deletion has been suggested to represent a sign of adaptation to humans [12]. Only four amino acid residues in the receptor glycoprotein ACE2-binding domain of the viral spike protein differ between the human epidemic SARS-CoV strains and civet strains, but they cause more than a 1000-fold difference in binding affinity to theACE2 molecule [13,14]. Antibodies to SARS-CoV spike (S) protein have been shown to prevent virus entry and neutralize virus infectivity in vitro [32,46]. Prophylactically administered monoclonal antibodies and passively transferred SARS-CoV hyper-immune sera have been shown to	Several groups have studied SARS-CoV infection in animals in the presence of neutralizing and sub-neutralizing levels of SARS-CoV anti-sera or anti SARS-CoV S-protein monoclonal antibodies, but no evidence of enhanced respiratory disease has been observed. However, foci of hepatic necrosis were noted following SARS-CoV challenge in MVA-SARS-S immunized ferrets [38]. It was strongly urged, therefore, that the experiment be repeated in ferrets. Additional experiments, in nonhuman primates and hamsters, looking for evidence of enhanced respiratory and hepatic diseases upon vaccination and challenge were also encouraged.	Vaccine. 2006 Nov 30;24(49-50):7056-65. Epub 2006 Jul 18. PMID: 16930781 DOI: 10.1016/j.vaccine.2006.07.009 Roberts A, Wood J, Subbarao K, Ferguson M, Wood D, Cherian T. Animal models and antibody assays for evaluating candidate SARS vaccines: summary of a technical meeting 25-26 August 2005, London, UK.

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			prevent SARS-CoV infection and associated disease following SARS-CoV challenge of naïve mice and hamsters [21,34,47–49]. Monoclonal antibodies administered therapeutically (i.e. post-infection) also have been shown to limit viral replication and reduce associated disease in hamsters [50].		
68	SARS, passive transfer and prior infection (no vaccine) (Subbarao 2004)	Mice	Following intranasal administration, the severe acute respiratory syndrome (SARS) coronavirus replicated to high titers in the respiratory tracts of BALB/c mice. Peak replication was seen in the absence of disease on day 1 or 2, depending on the dose administered, and the virus was cleared within a week. Viral antigen and nucleic acid were detected in bronchiolar epithelial cells during peak viral replication. Mice developed a neutralizing antibody response and were protected from reinfection 28 days following primary infection. Passive transfer of immune serum to naïve mice prevented virus replication in the lower respiratory tract following intranasal challenge. Thus, antibodies, acting alone, can prevent replication of the SARS coronavirus in the lung, a promising observation for the development of vaccines, immunotherapy, and immunoprophylaxis regimens.	No info	J Virol. 2004 Apr;78(7):3572-7. PMID:15016880 PMCID: PMC371090 DOI: 10.1128/jvi.78.7.3572-3577.2004 Subbarao K[1], McAuliffe J, Vogel L, Fahle G, Fischer S, Tatti K, Packard M, Shieh WJ, Zaki S, Murphy B. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice.

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69	SARS infection (no vaccine) (Roberts 2005)	Mice, aged BALB/c	Advanced age has repeatedly been identified as an independent correlate of adverse outcome and a predictor of mortality in cases of severe acute respiratory syndrome (SARS). SARS-associated mortality may exceed 50% for persons aged 60 years or older. Heightened susceptibility of the elderly to severe SARS and the ability of SARS coronavirus to replicate in mice led us to examine whether aged mice might be susceptible to disease. We report here that viral replication in aged mice was associated with clinical illness and pneumonia, demonstrating an age-related susceptibility to SARS disease in animals that parallels the human experience. Replication of SARS-CoV is enhanced and prolonged in 12- to 14-month-old BALB/c mice compared to that in young mice, and the enhanced viral replication is accompanied by evidence of clinical illness, alveolar damage, and interstitial pneumonitis. Elevation of proinflammatory cytokines is also observed in SARS infected, but not in mock-infected, aged mice.	No info	J Virol. 2005 May;79(9):5833-8. PMID:15827197 PMCID: PMC1082763 DOI: 10.1f128/JVI.79.9.5833-5838.200 Roberts A[1], Paddock C, Vogel L, Butler E, Zaki S, Subbarao K. Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans.

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70	SARS infection (no vaccine) (Chen 2010)	Mice, aged BALB/c	We characterized the cellular immune response to severe acute respiratory syndrome coronavirus (SARSCoV) infection in 12- to 14-month-old BALB/c mice, a model that mimics features of the human disease. Following intranasal administration, the virus replicated in the lungs, with peak titers on day 2 postinfection. Enhanced production of cytokines (tumor necrosis factor alpha [TNF-α] and interleukin-6 [IL-6]) and chemokines (CXCL10, CCL2, CCL3, and CCL5) correlated with migration of NK cells, macrophages, and plasmacytoid dendritic cells (pDC) into the lungs. By day 7, histopathologic evidence of pneumonitis was seen in the lungs when viral clearance occurred. At this time, a second wave of enhanced production of cytokines (TNF-α, IL-6, gamma interferon [IFN-γ], IL-2, and IL-5), chemokines (CXCL9, CXCL10, CCL2, CCL3, and CCL5), and receptors (CXCR3, CCR2, and CCR5), was detected in the lungs, associated with an influx of T lymphocytes. Depletion of CD8 T cells at the time of infection did not affect viral replication or clearance. However, depletion of CD4 T cells resulted in an enhanced immune-mediated interstitial pneumonitis and delayed clearance of SARS-CoV from the lungs, which was associated with reduced neutralizing antibody and cytokine production and reduced pulmonary recruitment of lymphocytes. Innate defense mechanisms are able to control SARS-CoV infection in the absence of CD4 and CD8 T cells and antibodies. Our findings provide new insights into the pathogenesis of SARS, demonstrating the important role of CD4 but not CD8 T cells in primary SARS-CoV infection in this model.	No info	J Virol. 2010 Feb;84(3):1289-301. doi: 10.1128/JVI.01281-09. Epub 2009 Nov 11. PMID:19906920 PMCID: PMC2812346 Chen J[1], Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, Subbarao K. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection.

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71	SARS hamster model (no vaccine) (Roberts 2005)	Hamsters, Golden Syrian	We investigated the ability of SARS CoV to infect 5-week-old Golden Syrian hamsters. When administered intranasally, SARS CoV replicates to high titers in the lungs and nasal turbinates. Peak replication in the lower respiratory tract was noted on day 2 postinfection (p.i.) and was cleared by day 7 p.i. Low levels of virus were present in the nasal turbinates of a few hamsters at 14 days p.i. Viral replication in epithelial cells of the respiratory tract was accompanied by cellular necrosis early in infection, followed by an inflammatory response coincident with viral clearance, focal consolidation in pulmonary tissue, and eventual pulmonary tissue repair. Despite high levels of virus replication and associated pathology in the respiratory tract, the hamsters showed no evidence of disease. Neutralizing antibodies were detected in sera at day 7 p.i., and mean titers at day 28 p.i. exceeded 1:400. Hamsters challenged with SARS CoV at day 28 p.i. were completely protected from virus replication and accompanying pathology in the respiratory tract. Comparing these data to the mouse model, SARS CoV replicates to a higher titer and for a longer duration in the respiratory tract of hamsters and is accompanied by significant pathology that is absent in mice. Viremia and extrapulmonary spread of SARS CoV to liver and spleen, which are seen in hamsters, were not detected in mice. The hamster, therefore, is superior to the mouse as a model for the evaluation of antiviral agents and candidate vaccines against SARS CoV replication.	Hamsters that had recovered from primary infection were protected from challenge with SARS CoV at day 28, as indicated by greatly decreased virus replication, a lack of detectable viral antigen, and the absence of pneumonitis. No vaccine data.	J Virol. 2005 Jan;79(1):503-11. PMID:15596843 PMCID: PMC538722 DOI: 10.1128/JVI.79.1.503-511.2005 Roberts A[1], Vogel L, Guarner J, Hayes N, Murphy B, Zaki S, Subbarao K. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters.

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72	SARS NHP model (no vaccines) (McAuliffe 2004)	NHP (rhesus, cynomolgus, African Green)	SARS coronavirus (SARS-CoV) administered intranasally and intratracheally to rhesus, cynomolgus and African Green monkeys (AGM) replicated in the respiratory tract but did not induce illness. The titer of serum neutralizing antibodies correlated with the level of virus replication in the respiratory tract (AGM>cynomolgus>rhesus). Moderate to high titers of SARS-CoV with associated interstitial pneumonitis were detected in the lungs of AGMs on day 2 and were resolving by day 4 post-infection. Following challenge of AGMs 2 months later, virus replication was highly restricted and there was no evidence of enhanced disease. These species will be useful for the evaluation of the immunogenicity of candidate vaccines, but the lack of apparent clinical illness in all three species, variability from animal to animal in level of viral replication, and rapid clearance of virus and pneumonitis in AGMs must be taken into account by investigators considering the use of these species in efficacy and challenge studies.	No evidence of enhanced disease after challenge at 2 mo. in AGM.	Virology. 2004 Dec 5;330(1):8-15. PMID:15527829 DOI: 10.1016/j.virol.2004.09.030 McAuliffe J, Vogel L, Roberts A, Fahle G, Fischer S, Shieh WJ, Butler E, Zaki S, St Claire M, Murphy B, Subbarao K. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys.

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73	MERS spike antigen (Pallesen 2017)	Mice, Female BALB/cJ	As in other coronaviruses, the spike (S) glycoprotein of MERS-CoV mediates receptor recognition and membrane fusion and is the primary target of the humoral immune response during infection. Here we use structure-based design to develop a generalizable strategy for retaining coronavirus S proteins in the antigenically optimal prefusion conformation and demonstrate that our engineered immunogen is able to elicit high neutralizing antibody titers against MERS-CoV. We also determined high resolution structures of the trimeric MERS-CoV S ectodomain in complex with G4, a stem-directed neutralizing antibody. The structures reveal that G4 recognizes a glycosylated loop that is variable among coronaviruses and they define four conformational states of the trimer wherein each receptor-binding domain is either tightly packed at the membrane-distal apex or rotated into a receptor accessible conformation. Our studies suggest a potential mechanism for fusion initiation through sequential receptor-binding events and provide a foundation for the structure-based design of coronavirus vaccines.	No info	Proc Natl Acad Sci U S A. 2017 Aug 29;114(35):E7348-E7357. doi: 10.1073/pnas.1707304114. Epub 2017 Aug 14. PMID: 28807998 PMCID: PMC5584442 Pallesen J[1], Wang N[2], Corbett KS[3], Wrapp D[4], Kirchdoerfer RN[1], Turner HL[1], Cottrell CA[1], Becker MM[5], Wang L[6], Shi W[6], Kong WP[6], Andres EL[5], Kettenbach AN[4,7], Denison MR[5,8], Chappell JD[5], Graham BS[3], Ward AB[9], McLellan JS[2]. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen.

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74	General MERS review (Memish 2020)	None	Excellent updated review. The Middle East respiratory syndrome coronavirus (MERS-CoV) is a lethal zoonotic pathogen that was first identified in humans in Saudi Arabia and Jordan in 2012. Intermittent sporadic cases, community clusters, and nosocomial outbreaks of MERS-CoV continue to occur. Between April 2012 and December 2019, 2499 laboratory-confirmed cases of MERS-CoV infection, including 858 deaths (34·3% mortality) were reported from 27 countries to WHO, the majority of which were reported by Saudi Arabia (2106 cases, 780 deaths). Large outbreaks of human-to-human transmission have occurred, the largest in Riyadh and Jeddah in 2014 and in South Korea in 2015. MERS-CoV remains a high-threat pathogen identified by WHO as a priority pathogen because it causes severe disease that has a high mortality rate, epidemic potential, and no medical countermeasures. This Seminar provides an update on the current knowledge and perspectives on MERS epidemiology, virology, mode of transmission, pathogenesis, diagnosis, clinical features, management, infection control, development of new therapeutics and vaccines, and highlights unanswered questions and priorities for research, improved management, and prevention.	No Info	Lancet. 2020 Mar 28;395(10229):1063-1077. doi: 10.1016/S0140-6736(19)33221-0. Epub 2020 Mar 4. PMID:32145185 Memish ZA[1], Perlman S[2], Van Kerkhove MD[3], Zumla A[4]. Middle East respiratory syndrome.

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
75	Structure SARS-CoV-2 Spike (no vaccine) (Walls 2020)	None, cell lines	We show that SARS-CoV-2 S uses ACE2 to enter cells and that the receptor-binding domains of SARS-CoV-2 S and SARS-CoV S bind with similar affinities to human ACE2, correlating with the efficient spread of SARSCoV-2 among humans. We found that the SARSCoV-2 S glycoprotein harbors a furin cleavage site at the boundary between the S1/S2 subunits, which is processed during biogenesis and sets this virus apart from SARS-CoV and SARS-related CoVs. We determined cryo-EM structures of the SARSCoV-2 S ectodomain trimer, providing a blueprint for the design of vaccines and inhibitors of viral entry. Finally, we demonstrate that SARS-CoV S murine polyclonal antibodies potently inhibited SARSCoV-2 S mediated entry into cells, indicating that cross-neutralizing antibodies targeting conserved S epitopes can be elicited upon vaccination.	No info	Cell. 2020 Mar 6. pii: S0092-8674(20)30262-2. doi: 10.1016/j.cell.2020.02.058. [Epub ahead of print] PMID:32155444 Walls AC[1], Park YJ[1], Tortorici MA[2], Wall A[3], McGuire AT[4], Veesler D[5]. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein.

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
76	MERS moAb (no vaccine) (de Wit 2019)	NHP, common marmoset	Effective antiviral treatments for MERS-CoV are urgently needed. LCA60 is a MERS-CoV-neutralizing monoclonal antibody isolated from a convalescent MERS patient. Previously, it was shown that treatment with LCA60 resulted in reduced disease and virus titers in mouse models of MERS-CoV infection. Here, we tested the prophylactic efficacy of LCA60 in the common marmoset model of MERS-CoV infection. Intravenous administration of LCA60 one day before virus challenge resulted in high levels of MERS-CoV-neutralizing activity in circulating blood. Clinically, there was a moderate benefit of treatment with LCA60 including reduced respiratory involvement. Although viral lung loads were not reduced in LCA60-treated animals as compared to controls, there were fewer pathological changes in the lungs. Thus, prophylactic LCA60 treatment could be implemented to reduce disease burden in contacts of confirmed MERS-CoV patients.	Not seen although moAb given 1 day prior to challenge and only moderate clinical benefit with no effect virologically.	Antiviral Res. 2019 Mar;163:70-74. doi: 10.1016/j.antiviral.2019.01.016. Epub 2019 Jan 24. PMID:30684561 de Wit E, Feldmann F, Horne E, Okumura A, Cameroni E, Haddock E, Saturday G, Scott D, Gopal R, Zambon M, Corti D, Feldmann H. Prophylactic efficacy of a human monoclonal antibody against MERS-CoV in the common marmoset.

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77	SARS-CoV-2 moAb (no vaccine) (Wang 2020)	None, in vitro studies	Here we report a human monoclonal antibody that neutralizes SARS-CoV-2 (and SARS-CoV). This cross-neutralizing antibody targets a communal epitope on these viruses and offers potential for prevention and treatment of COVID-19. Human 47D11 antibody binds to cells expressing the full-length spike proteins of SARS-CoV and SARS-CoV-2 and was found to potently inhibit infection of VeroE6 cells with SARS-S and SARS2-S pseudotyped VSV with IC50 values of 0.06 and 0.08 µg/ml. Our data show that 47D11 neutralizes SARS-CoV and SARS-CoV-2 through a yet unknown mechanism that is different from receptor binding interference. Alternative mechanisms of coronavirus neutralization by RBD-targeting antibodies have been reported including spike inactivation through antibody-induced destabilization of its prefusion structure, which may also apply for 47D11. This is the first report on a (human) monoclonal antibody that neutralizes SARS-CoV-2.	No info	bioRxiv. posted 12 March 2020, 10.1101/2020.03.11.987958 http://biorxiv.org/content/early/2020/03/12/2020.03.11.987958 Wang C, Li W, Drabek D, Okba N, van Haperen R, Osterhaus A, van Kuppeveld F, Haagmans BL, Grosveld F and Bosch B-J. A human monoclonal antibody blocking SARS-CoV-2 infection.
78	SARS-CoV-2 isolate experimental infection for pathogenesis (no vaccine) (Deng 2020)	NHP, rhesus macaques	In some clinical cases, samples of tears and conjunctival secretions from both SARS-CoV[1] and SARS-CoV-2 patients with conjunctivitis[2] displayed detectable viral RNA. A previous study reported the case of a clinician who was infected with SARS-CoV-2 while working with patients under all safeguards except eye protection [2]. By contrast, no SARS-CoV-2 could be detected by RT-PCR in 114 conjunctival swabs samples from patients with COVID-19 pneumonia [4]. Anatomically, the linkage of the ocular with respiratory tissues is primarily by the nasolacrimal system [5].	No info	bioRxiv. posted 14 March 2020, 10.1101/2020.03.13.990036 Wei Deng, Linlin Bao, Hong Gao, Zhiguang Xiang, Yajin Qu, Zhiqi Song, Shunran Gong, Jiayi Liu, Jiangning Liu, Pin Yu, Feifei Qi, Yanfeng Xu, Fengli Li, Ch

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			Three rhesus macaques between the ages of 3 and 5 years were inoculated with 1×106 50% tissue-culture infectious doses (TCID50) of SARS-CoV-2. Two of them were randomly selected to apply for ocular conjunctival inoculation, the remaining one was inoculated via intratracheal route as a comparison to compare the distribution and pathogenesis of viruses in infected-host via different routes. Results suggest that conjunctiva is a portal for viral transmission. In our results, viral load can be detectable in several nasolacrimal system associated-tissues, especially in the conjunctiva, lacrimal gland, nasal cavity and throat, which outlined the anatomical bridge between ocular and respiratory tissues. Particularly, the lacrimal duct functions as a conduit to collect and transport tear fluid from the ocular surface to the nasal-inferior meatus, being convenient for the drainage of the virus from ocular to respiratory tract tissues.		ong Xiao, Qi Lv, Jing Xue, Qiang Wei, Mingya Liu, Guanpeng Wang, Shunyi Wang, Haisheng Yu, Xing Liu, Wenjie Zhao, Yunlin Han, Chuan Qin Rhesus macaques can be effectively infected with SARS-CoV-2 via ocular conjunctival route
79	SARS-CoV-2 challenge, (no vaccine) (Bao 2020)	NHP, rhesus macaques	Recently, it has been reported that discharged patients in China and elsewhere were testing positive after recovering. However, it remains unclear whether the convalescing patients have a risk of “relapse” or “reinfection”. The longitudinal tracking of re-exposure after the disappeared symptoms of 4 SARS-CoV-2-infected monkeys was performed in this study. We found that weight loss in some monkeys, viral replication mainly in nose, pharynx, lung and gut, as well as moderate interstitial pneumonia at 7 days post-infection (dpi) were clearly observed in rhesus monkeys after the primary infection. After the symptoms were alleviated and the specific antibody tested positively, half of infected monkeys were rechallenged with the same dose of SARS-CoV-2 strain. Notably, neither viral loads in nasopharyngeal and anal swabs along timeline nor viral replication in all primary tissue compartments at 5 days post-reinfection (dpr) was found in re-exposed monkeys. Combined with the follow-up virologic, radiological and pathological findings, the monkeys with re-exposure showed no	No disease seen on rechallenge so no evidence of enhancement but interval was only 28 days. The titers of 1:16 (M2, M4) and 1:8 (M3) exhibited the neutralizing effect at 21 dpi and 28 dpi. After the re-exposure, the titers for M4 elevated 1:40 at 5 dpr and 14 dpr, while M3 maintained the same titer at 1:8 at 5 dpr. In this study, ADE was not found in infected monkeys that were subsequently exposed to SARS-CoV-2.	bioRxiv. posted 14 March 2020, 10.1101/2020.03.13.990226 http://biorxiv.org/content/early/2020/03/14/2020.03.13.990226 Linlin Bao, Wei Deng, Hong Gao, Chong Xiao, Jiayi Liu, Jing Xue, Qi Lv, Jiangning Liu, Pin Yu, Yanfeng Xu, Feifei Qi, Yajin Qu, Fengdi Li, Zhiguang Xiang, Haisheng Yu, Shura

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			recurrence of COVID-19, similar to the infected monkey without rechallenge. Taken together, our results indicated that the primary SARS-CoV-2 infection could protect from subsequent exposures (28 days later), which have the reference of prognosis of the disease and vital implications for vaccine design.		n Gong, Mingya Liu, Guanpeng Wang, Sh unyi Wang, Zhiqi Son g, Wenjie Zhao, Yunli n Han, Linna Zhao, Xi ng Liu, Qiang Wei, Ch uan Qin Reinfection could not occur in SARS-CoV-2 infected rhesus macaques
80	SARS-CoV-2 plasma (Lv 2020)	In vitro studies Mice	One major immunological question is concerning the antigenic differences between SARS-CoV-2 and SARS-CoV. We address this question by using plasma from patients infected by SARS-CoV-2 or SARS-CoV, and plasma obtained from infected or immunized mice. Our results show that while cross-reactivity in antibody binding to the spike protein is common, cross-neutralization of the live viruses is rare, indicating the presence of non-neutralizing antibody response to conserved epitopes in the spike. Whether these non-neutralizing antibody responses will lead to antibody-dependent disease enhancement needs to be addressed in the future. Fifteen heparin anticoagulated plasma samples (from day 2 to 22 post-symptom onset) from SARS-CoV-2 infected patients were analyzed. As compared to the plasma from healthy donors, plasma from patients from day 10 post-symptom onward reacted strongly in ELISA binding assays to the S ectodomain and RBD of SARS-CoV-2. The plasma from SARS-CoV-2-infected patients could also cross-react, although less strongly, with the SARS-CoV S ectodomain and the SARS-CoV RBD. Only five of the samples from the SARS-CoV-2-infected patients had convincing antibody binding responses to the SARS-CoV RBD. The other plasma reacted more weakly or not at all with the SARS-CoV	Non-neutralizing antibody responses can also lead to antibody-dependent enhancement (ADE) of infection as reported in other coronaviruses (Tseng et al., 2012; Wang et al., 2014; Weiss and Scott, 1981). Whether ADE plays a role in SARS-CoV-2 infection will need to be carefully examined, due to its potential adverse effect in vaccination (Tseng et al., 2012).	Cell Rep. 2020 Jun 2;31(9):107725. doi: 10.1016/j.celrep.202 0.107725. Epub 2020 May 18. PMID: 32684246 Lv H, Wu NC, Tsang OT, Yuan M, Perera RAPM, Leung WS, So RTY, Chan JMC, Yip GK, Chik TSH, Wang Y, Choi CYC, Lin Y, Ng WW, Zhao J, Poon LLM, Peiris JSM, Wilson IA, Mok CKP. Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			RBD. This result indicates that the cross-reactive antibody response to the S protein after SARS-CoV-2 infection mostly targets non-RBD regions. Next tested the neutralization activity of plasma samples from SARS-CoV-2- infected patients; except for four plasma samples that came from patients with less than 12 days post-symptom onset with concomitantly low reactivity to both SARS-CoV-2 S ectodomain and RBD, all other plasma samples could neutralize the SARS-CoV-2 virus with titers ranging from 1:40 to 1:640. Only one plasma sample could cross-neutralize SARS-CoV, with very low neutralization activity (1:10). While cross-reactive antibody binding responses to both SARS-CoV-2 and SARS-CoV S 172 proteins appears to be relatively common in this cohort, cross-neutralizing responses are rare. Only one out of 15 SARS-CoV-2-infected patients was able to generate a cross neutralizing response to both SARS-CoV-2 and SARS-CoV viruses, and this cross reactive response was very weak. Therefore, it is possible that only a subset of the cross-reactive binding epitopes is a bona fide neutralizing epitope.		
81	SARS, MERS and SARS-CoV-2 (Rockx 2020)	NHP, cynomolgus	A novel coronavirus, SARS-CoV-2, was recently identified in patients with an acute respiratory syndrome, COVID-19. To compare its pathogenesis with that of previously emerging coronaviruses, we inoculated cynomolgus macaques with SARS-CoV-2 or MERS-CoV and compared with historical SARS-CoV infections. In SARS-CoV-2-infected macaques, virus was excreted from nose and throat in absence of clinical signs, and detected in type I and II pneumocytes in foci of diffuse alveolar damage and mucous glands of the nasal cavity. In SARS-CoV-infection, lung lesions were typically more severe, while they were milder in MERS-CoV infection, where virus was detected mainly in type II pneumocytes. These data show that SARS-CoV-2 can cause a COVID-19-like disease, and suggest that the severity of SARS-CoV-2 infection is intermediate between that of SARS-CoV and MERS-CoV.	No info	Science. 2020 May 29;368(6494):1012-1015. doi: 10.1126/science.abb7314. Epub 2020 Apr 17.PMID: 32303590 Rockx B, Kuiken T, Herfst S, Bestebroer T, Lamers MM, Oude Munnink BB, de Meulder D, van Amerongen G, van den Brand J, Okba NMA, Schipper D, van Run P, Leijten L,

#	VACCINE(S) (1ST AUTHOR YR PUBLISHED)	ANIMAL MODEL	FINDINGS	ENHANCED DISEASE ON CHALLENGE POST-VACCINATION?	REFERENCE
			SARS-CoV-2 infection in macaques results in COVID-19-like disease with prolonged virus excretion from nose and throat in absence of clinical signs.		Sikkema R, Verschoor E, Verstrepen B, Bogers W, Langermans J, Drosten C, Fentener van Vlissingen M, Fouchier R, de Swart R, Koopmans M, Haagmans BL Comparative Pathogenesis Of COVID-19, MERS And SARS In A Non-Human Primate Model

APPENDIX 5: CONSENSUS SUMMARY REPORT FOR CEPI/BC MARCH 12-13, 2020 MEETING: ASSESSMENT OF RISK OF DISEASE ENHANCEMENT WITH COVID-19 VACCINES

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Conference report

Consensus summary report for CEPI/BC March 12–13, 2020 meeting: Assessment of risk of disease enhancement with COVID-19 vaccines

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ABSTRACT

A novel coronavirus (CoV), Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in late 2019 in Wuhan, China and has since spread as a global pandemic. Safe and effective vaccines are thus urgently needed to reduce the significant morbidity and mortality of Coronavirus Disease 2019 (COVID-19) disease and ease the major economic impact. There has been an unprecedented rapid response by vaccine developers with now over one hundred vaccine candidates in development and at least six having reached clinical trials. However, a major challenge during rapid development is to avoid safety issues both by thoughtful vaccine design and by thorough evaluation in a timely manner. A syndrome of "disease enhancement" has been reported in the past for a few viral vaccines where those immunized suffered increased severity or death when they later encountered the virus or were found to have an increased frequency of infection. Animal models allowed scientists to determine the underlying mechanism for the former in the case of Respiratory syncytial virus (RSV) vaccine and have been utilized to design and screen new RSV vaccine candidates. Because some Middle East respiratory syndrome (MERS) and SARS-CoV-1 vaccines have shown evidence of disease enhancement in some animal models, this is a particular concern for SARS-CoV-2 vaccines. To address this challenge, the Coalition for Epidemic Preparedness Innovations (CEPI) and the Brighton Collaboration (BC) Safety Platform for Emergency vACcines (SPEAC) convened a scientific working meeting on March 12 and 13, 2020 of experts in the field of vaccine immunology and coronaviruses to consider what vaccine designs could reduce safety concerns

Abbreviations: ACE2, Angiotensin-converting enzyme 2; ADE, Antibody disease enhancement; ARDS, Acute respiratory distress syndrome; B/HPIV3, Bovine/human parainfluenza virus type 3; BC, Brighton Collaboration; BPL, β-Propiolactone; BtCoV, Bat coronavirus; CEPI, Coalition for Epidemic Preparedness Innovations; CNS, Central nervous system; COVID-19, Coronavirus Disease 2019; CRISPR, Clustered regularly interspaced short palindromic repeats; DNA, Deoxyribonucleic acid; DPP4, Dipeptidyl peptidase-4; hACE2, Human ACE2 receptor; HBs, Hepatitis B surface antigen; hDPP4, Human DPP4; IHC, Immunohistochemistry; MERS CoV, Middle East respiratory syndrome coronavirus; mRNA, Messenger RNA; MVA, Modified Vaccinia Virus Ankara; NHP, Non-human primate; Non-SPF, Non-specific pathogen free; NTD, N terminal domain; RAG1, Recombination activating gene 1; RBD, Receptor binding domain; rMVA, Recombinant modified vaccinia virus Ankara; RNA, Ribonucleic acid; RSV, Respiratory syncytial virus; SARS-CoV-1, Severe acute respiratory syndrome coronavirus 1; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SPEAC, Safety Platform for Emergency vACcines; TCR, T-cell receptor; Tg, Transgenic; Th1, T-helper cell type 1; Th2, T-helper cell type 2; VSV, Vesicular stomatitis virus; WHO, World Health Organization.

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Consensus Summary Report for CEPI/BC March 12-13, 2020 Meeting: Assessment of Risk of Disease Enhancement with COVID-19 Vaccines

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Abstract

A novel coronavirus (CoV), Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in late 2019 in Wuhan, China and has since spread as a global pandemic. Safe and effective vaccines are thus urgently needed to reduce the significant morbidity and mortality of Coronavirus Disease 2019 (COVID-19) disease and ease the major economic impact. There has been an unprecedented rapid response by vaccine developers with now over one hundred vaccine candidates in development and at least six having reached clinical trials. However, a major challenge during rapid development is to avoid safety issues both by thoughtful vaccine design and by thorough evaluation in a timely manner. A syndrome of "disease enhancement" has been reported in the past for a few viral vaccines where those immunized suffered increased severity or death when they later encountered the virus or were found to have an increased frequency of infection. Animal models allowed scientists to determine the underlying mechanism for the former in the case of Respiratory Syncytial virus (RSV) vaccine and have been utilized to design and screen new RSV vaccine candidates. Because some Middle East respiratory syndrome (MERS) and SARS-CoV-1 vaccines have shown evidence of disease enhancement in some animal models, this is a particular concern for SARS-CoV-2 vaccines. To address this challenge, the Coalition for Epidemic Preparedness Innovations (CEPI) and the Brighton Collaboration (BC) Safety Platform for Emergency vACcines (SPEAC) convened a scientific working meeting on March 12 and 13, 2020 of experts in the field of vaccine immunology and coronaviruses to consider what vaccine designs could reduce safety concerns and how animal models and immunological assessments in early clinical trials can help to assess the risk. This report summarizes the evidence presented and provides considerations for safety assessment of COVID-19 vaccine candidates in accelerated vaccine development.

Keywords: SARS-CoV-2, COVID-19, vaccine safety, MERS-CoV vaccine, SARS-CoV-1 vaccine, SARS-CoV-2 vaccine, animal models, enhanced disease, vaccine adjuvants

Abbreviations

ACE2	Angiotensin-converting enzyme 2
ADE	Antibody disease enhancement
ARDS	Acute respiratory distress syndrome
B/HPIV3	Bovine/human parainfluenza virus type 3
BC	Brighton Collaboration
BPL	β-Propiolactone
BtCoV	Bat coronavirus
CEPI	Coalition for Epidemic Preparedness Innovations
CNS	Central nervous system
COVID-19	Coronavirus Disease 2019
CRISPR	Clustered regularly interspaced short palindromic repeats
DNA	Deoxyribonucleic acid
DPP4	Dipeptidyl peptidase-4
hACE2	Human ACE2 receptor
HBs	Hepatitis B surface antigen
hDPP4	Human DPP4
IHC	Immunohistochemistry
MERS CoV	Middle East respiratory syndrome coronavirus
mRNA	Messenger RNA
MVA	Modified Vaccinia Virus Ankara
NHP	Non-human primate
Non-SPF	Non-specific pathogen free
NTD	N terminal domain
RAG1	Recombination activating gene 1
RBD	Receptor binding domain
rMVA	Recombinant modified vaccinia virus Ankara
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
SARS-CoV-1	Severe acute respiratory syndrome coronavirus 1
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SPEAC	Safety Platform for Emergency vACcines
TCR	T-cell receptor
Tg	Transgenic
Th1	T-helper cell type 1
Th2	T-helper cell type 2
VSV	Vesicular stomatitis virus
WHO	World Health Organization

1. Introduction

Since the identification of a novel coronavirus, SARS-CoV-2, as the cause of pneumonia in patients from Wuhan China, a pandemic has erupted, resulting in enormous health care, social and economic disruption to our global society [1]. As of May 17, 2020 there have been 4,708,415 cases and 314,950 deaths worldwide [2]. In rapid response to the pandemic, academic and industry scientists from around the world have initiated efforts to develop vaccines and therapeutics for disease prevention and patient management. The Coalition for Epidemic Preparedness Innovations (CEPI), a global partnership between public, private, philanthropic, and civil organizations, is funding work to develop SARS-CoV-2 vaccines using a variety of technology platforms. Several vaccine candidates are already in Phase 1 studies with others likely to enter the clinic in the next few months [3].

One of the challenges facing rapid vaccine development for SARS-CoV-2 is the need to adequately assure the safety of these vaccines. One such safety concern is disease enhancement syndrome that occurred in the 1960s with inactivated RSV and measles vaccines. Vaccine-mediated disease enhancement is characterized by a vaccine that results in increased disease severity if the subject is later infected by the natural virus. During early trials with inactivated RSV vaccine, the vaccine did not prevent infection, 80% of those infected required hospitalization and two children died [4]. Lung pathology in patients showed an unexpected inflammatory response with both neutrophils and eosinophils, evidence of immune complex formation and complement activation in small airways [5]. Scientists later learned that the vaccine caused a similar disease enhancement in animals characterized by immunopathology and a T helper cell type 2 (Th2) biased response and antibody responses with poor neutralizing activity [6-8]. Since that time, the animal models have been relied upon to predict safety for new RSV vaccines that are developed. Of note, the pathogenesis of RSV disease enhancement is distinct from antibody disease enhancement (ADE) which occurs for macrophage tropic viruses, demonstrated most notably for Dengue in humans and the coronavirus feline infectious peritonitis virus in cats, and is directly caused by non-neutralizing or sub-neutralizing antibodies leading to more efficient viral uptake via Fcγ receptor binding [9].

Since pathology consistent with the RSV vaccine enhanced disease (and perhaps ADE) has been demonstrated for some SARS-CoV-1 vaccine candidates in animal models, there is also a concern that a similar syndrome could occur in humans immunized with SARS-CoV-2 candidate vaccines. Therefore, CEPI and the Brighton Collaboration Safety Platform for Emergency vACcines (SPEAC) convened a scientific working meeting https://brightoncollaboration.us/brighton-collaboration-cepi-covid-19-web-conference/) on March 12 and 13, 2020 of experts in the field of vaccine immunology and coronaviruses to discuss current knowledge that could form the basis for the assessment of the risk of enhanced disease during SARS-CoV-2 vaccine development. This consensus report presents considerations for vaccine developers and can serve as a guide for the development and testing of vaccine candidates to avoid these safety concerns. Ultimately, the door to clinical trials is controlled by regulators in the context of the risk/benefit for the entire dataset provided by developers and within the local trial context.

2. Animal Models of SARS-CoV-1 And MERS CoV

Dr. Kanta Subbarao, director of the WHO Collaborating Centre for Reference and Research on Influenza and Professor in the Department of Microbiology and Immunology at the University of Melbourne, and Dr. Stanley Perlman, Professor in the Departments of Microbiology and Immunology and Pediatrics at the University of Iowa, both reviewed their work and that of others in animal models developed for SARS-CoV-1 and MERS-CoV. The lessons from these models can inform the development priorities for useful SARS-CoV-2 animal models to address both efficacy and safety.

In inbred mouse strains, SARS-CoV-1 replicates efficiently in the respiratory tract and can cause pneumonitis, but clinical signs and pneumonia were only observed in old BALB/c mice [10]. Subsequent passage of SARS-CoV-1 through mouse lungs resulted in the isolation of virus that caused severe disease in both young and old mice [11, 12]. This virus was used in many subsequent studies. Ferret models of SARS-CoV-1 also demonstrate virus replication in respiratory tracts with induction of a neutralizing antibody response but also demonstrated little evidence of clinical disease [13]. Hamsters, in contrast to mice and ferrets, demonstrate high levels of viral replication, develop pneumonitis, and can be shown to have clinical signs of disease [14]. Following the identification of human ACE2 as the receptor for SARS-CoV-1, transgenic murine models expressing human ACE2 receptor (hACE2) were developed and shown to develop mild pulmonary disease. Of note, these mice also developed lethal viral encephalitis, attributed to viral spread through the olfactory nerve, despite the relative scarcity of hACE2 expression in the brain which may have relevance to SARS-CoV-2 disease [15].

Efficacy of several SARS-CoV-1 vaccines was evaluated in these models with spike (S) protein based vaccines demonstrating neutralizing antibody and protection against pulmonary replication of the challenge virus in mice and hamsters [16]. For DNA vaccine studies, it was shown that candidate vaccines encoding the S protein conferred antibody mediated protection from challenge in mice and that vaccines encoding the N protein induced humoral and cellular immunity [17, 18]. For vectored vaccines expressing SARS-CoV-1 proteins, it was shown that viral proteins were expressed in mice, ferrets, and hamsters. In these studies, neutralizing antibodies were elicited by B/HPIV3, VSV, rabies, MVA and adeno viruses expressing S protein, that protected against SARS-CoV-1 replication in lungs of challenged animals. However, one MVA vaccine expressing the S-protein did not protect against infection [16].

In contrast to SARS-CoV-1, inbred mice were found to be resistant to MERS-CoV, thus infection was studied by creating models that expressed the MERS receptor, human DPP4 (hDPP4). Ad5-hDPP4 transduced mice could be infected with MERS virus but infection was associated with minimal clinical disease except in immunocompromised mice that developed weight loss after infection. Of note, hDPP4-transgenic mice developed lethal viral encephalitis with concurrent inflammatory changes on histopathological examination of the lung, similar to hACE2-Tg mice with SARS-CoV-1. Subsequently, investigators developed mice "knocked-in" for expression of hDPP4 and after virus passage in these mice, identified mouse-adapted MERS strains that caused more severe disease and increased histopathology with more pulmonary edema than those infected with the original MERS strain [19]. Importantly, mice without functional T cells, such as RAG1-/- and TCR alpha-/-, had delayed viral clearance whereas mice that could not produce antibodies, muMT mice, did not show delay in clearance. Similar models were developed by CRISPR/Cas9 mutagenesis of two residues in the mouse ACE2 molecule, followed by mouse adaptation with serial passage, leading to an ARDS model of lethal infection [20, 21]. Taken together this evidence supports the notion that T cells are important in viral clearance for MERS [22].

Non-human primate (NHP) models have also been established for both SARS-CoV-1 and MERS-CoV. There was evidence of upper respiratory and lower respiratory tract SARS-CoV-1 replication in African green monkeys to a greater extent than in cynomolgus macaques, and least in rhesus macaques, with little evidence of clinical disease in all three species [23]. Of note, consistent with findings in older humans and mice, increased pathology has been documented in aged cynomolgus macaques with SARS-CoV-1 wild type infection [24]. There is some controversy on the disease severity in the MERS models with different groups seeing different levels of pathology. This has not been resolved [25, 26].

3. Enhanced Disease Following SARS-CoV-1 Vaccines

Both vaccine efficacy and safety have been studied in animal models with many SARS-CoV-1 candidate vaccines. The group of experts discussed how the vaccine models were utilized to characterize the response

of specific vaccines and to examine both disease enhancement and antibody dependent enhancement (ADE) signals.

There is evidence for disease enhancement in vaccinated animals after challenge with live virus in multiple studies with SARS–CoV-1 vaccine candidates as summarized in Table. We are limiting our comments in this report to data in animal models and not discussing in vitro data except to mention that there is some evidence of ADE in human primary monocytes [27, 28]. Different animal models exhibit different pulmonary pathology but generally characterized by cellular infiltrates including eosinophils. In this summary, we provide an overview of the consensus opinion on vaccine related outcomes in animal models that were of concern for risk of disease enhancement and could guide assessments of SARS-CoV-2 vaccine candidates.

In murine models, evidence for vaccine related disease enhancement has been demonstrated for inactivated whole vaccine (with and without alum), vectored vaccine expressing N protein (but not seen with vectored vaccine expressing S protein in same report), a replicon particle platform expressing S protein, and a vectored vaccine expressing S proteins. In general, the pathology described included pulmonary infiltrates often with eosinophils observed. Th2 dominant responses were documented in some reports by expression of Th2 driven cytokines [29-33]. In a ferret model, hepatitis was demonstrated in animals vaccinated with a recombinant modified vaccinia virus Ankara vaccine expressing S protein and then challenged with virus [34] although questions have been raised about this study [35].

Table: Evidence of enhanced disease in SARS-CoV-1 vaccine candidates

ANIMAL MODEL	VACCINE	ADJUVANT	IMMUNOPATHOLOGY	REFERENCE
Murine[1]	VEE Replicon Particles expressing N protein	-	YES	Deming 2006
Murine[2]	Recombinant Vaccinia virus expressing N protein	-	YES	Yasui 2008
Murine[3]	Inactivated Whole Virus	Alum	YES	Bolles 2011
		-	YES	
Murine[4]	Replicon Particles expressing S protein	-	YES	Sheahan 2011
Murine[5]	Inactivated Whole Virus and S protein vaccines	Alum	YES	Tseng 2012
		-	YES	
Ferret[6]	Recombinant Modified Vaccinia Virus Ankara (rMVA) expressing S protein	-	YES[†]	Weingartl 2004
NHP[7]	Modified Vaccinia Ankara (MVA) virus encoding full-length S protein	-	YES	Liu 2019
	Passive anti-S sera	N/A	YES	
NHP[7]	Inactivated Whole Virus	-	YES	Wang 2016/2020
	Passive Human SARS Antiserum	N/A	YES	

[1] Young and senescent female BALB/c mice
[2] BALB/c mice
[3] Aged BALB/c mice
[4] Young and aged BALB/c mice
[5] Female BALB/c mice
[6] Mustela putorius furo, castrated males
[7] Chinese rhesus macaque
[†] Acute injury

Of note, mouse models have also shown evidence of enhanced disease for inactivated and recombinant adenovirus 5-based MERS-CoV vaccine [36, 37].
Non-human primate models have also produced evidence of enhanced disease after SARS-CoV-1 vaccine immunization. Chinese macaques immunized with a modified vaccinia virus expressing S protein then challenged with SARS-CoV-1 did not develop clinical disease, but histopathology showed lung injury. This injury was characterized by decreased wound healing, and increased pro-inflammatory macrophages expressing IL-6, IL-8, and CCL2 [38]. This report also demonstrated that passively administered anti-S antibody was associated with lung pathology after challenge with the live virus although the mechanism may not be through Fc receptor and thus not classic "ADE". Of note, a second report similarly demonstrates the effect with certain anti-S antibody preparations and without Fc involvement [39, 40]. The relevance of these reports remains unclear as there are multiple studies with administration of neutralizing monoclonal antibodies to different models that did not induce disease enhancement. Other investigators have reported absence of disease enhancement in both hamsters and monkeys immunized with a whole inactivated vaccine although these models differed in a number of ways, most notably by the use of BPL (β-Propiolactone) instead of formalin for inactivation of the virus [41, 42]. Finally, we note that there has not be an agreed upon positive control applied in these animal studies and thus interpretations are hampered.ba

4. SARS-CoV-2 Murine and NHP Models Newly Developed

Animal models with SARS-CoV-2 are being rapidly developed by multiple research groups. Dr. Qin Chuan, Professor and Director of the Institute of Laboratory Animal Science, Comparative Medicine Center of the Peking Union Medical College presented data on SARS-CoV-2 infection in both transgenic mice and rhesus macaque models.
Human ACE2 transgenic mice (hACE2 Tg) aged 4-6 weeks and 6-11 months of age were studied and hACE2 expression was observed in lung, heart, kidney and intestinal tissues. Following intranasal inoculation with SARS-CoV-2, weight loss was observed, and viral RNA was detected in the lungs as well as in the intestine [43]. Gross pathology demonstrated swollen and enlarged lungs with moderate interstitial pneumonia. Histological studies documented an accumulation of inflammatory cells including monocytes and lymphocytes in alveolar interstitium, with thickening of alveolar walls. SARS-CoV-2 S protein was detected by IHC in alveolar macrophages and epithelia [43].
NHP were also infected with SARS-CoV-2 with 3 rhesus macaques aged 3-4 years inoculated intratracheally and although no fever was observed, weight loss and asthenia were seen on multiple days. Viral RNA was detected from nasal and throat swabs and to a lesser degree in anal specimens, peaking on days 3 to 7 and lasting until day 11 post infection. One animal was euthanized on day 7 for necropsy and viral RNA was detected in multiple organs
including CNS, skeletal muscle and heart. For the two surviving rhesus macaques, positive neutralization titers were documented by day 11 post infection. There was radiographic evidence of multiple ground glass opacities in the lungs on days 3, 5 and 7 post infection. Microscopically the lung lesions represented an acute interstitial pneumonia characterized by mild to moderate
thickening of alveolar septum, increased number of macrophages, degeneration of pneumocytes and an inflammatory cell infiltration. Presence of viral antigen was confirmed, predominately in alveolar monocytes and macrophages [44]. Analysis of blood samples showed a decline in counts of total white blood cells, lymphocytes and monocytes with no observed changes in percentages. A decrease in both CD3+CD4+ and CD3+ CD8+ T-cell counts was observed. Importantly, these hematological findings are similar to those seen in SARS-CoV-2 infected patients.
This model could serve as a critical tool for detailed studies of pathogenesis and the evaluation of intervention strategies including vaccines. Of note, following the meeting another group has confirmed SARS-CoV-2

infection in rhesus macaques with viral antigen detected in type I and type II pneumocytes and diffuse pulmonary alveolar damage noted [45]. Experts agreed that these models and others under development should be utilized to evaluate vaccine candidates for any evidence of disease enhancement as specified in later sections.

5. COVID-19 Vaccine Design Considerations for Efficacy and Safety

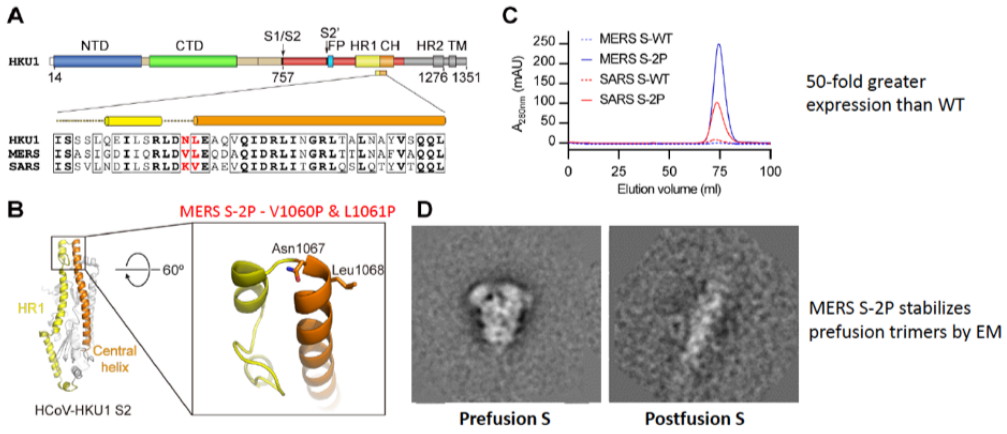
5.1 Structure and Function of S Glycoproteins in Coronavirus

Design of safe and effective COVID-19 vaccines can be informed by knowledge of previous coronavirus vaccine development activities and shared elements of viral pathogenesis for non-coronaviruses such as RSV. Specific epitope targets for potent neutralizing antibody, platforms for inducing both neutralizing antibody and effective T cell responses, and adjuvants for improving immunogenicity were presented at the conference. We review first the structure and function of the major target of COVID-19 vaccines, spike (S) glycoprotein.
Ralph Baric PhD, Professor in the Department of Epidemiology at the University of North Carolina Chapel Hill School of Medicine presented a review of the structure and function of coronavirus (CoV) S glycoprotein highlighting priorities for the development of vaccine and immune therapeutics. There is a long history of emerging CoVs with acceleration of cross-species movement and emergence of highly pathological strains in the last 16 years, including SARS-CoV-1, MERS-CoV, and SARS-CoV-2, and this trend is likely to increase in the future. Phylogenetic relationships within CoVs have been established, and Group 2B includes SARS-CoV-1 and SARS-like CoVs including SARS-CoV-2, BtCoV WIV1 and BtCoV SHC014. Similarly, Group 2C are MERS-like CoVs which are also poised for human emergence. Within Group 2B, known SARS-like CoVs are divided into high or low pre-epidemic potential. High risk features include use of ACE2 for cell entry, growth in primary human airway cells, causing ARDS, causing age-related disease severity, and escape from existing immune therapeutics. Drivers of CoV evolution include the high mutation rate of the RNA-dependent RNA polymerase paired with the regulated fidelity complex. CoVs also demonstrate high rates of RNA recombination as during mixed infection up to 25% of progeny are recombinant, and modular evolution allows CoVs to swap whole genes or portions of key proteins between strains. The S protein itself, which regulates host range, tissue tropism, and transmissibility, can tolerate a high mutation rate while retaining its function.
The organization of the SARS-CoV-2 genome has been elucidated and SARS-CoV-2, like SARS-CoV-1, has been shown to use hACE2 for cell entry. Group 2B viruses have fourteen contact interfaces between their S protein and ACE2. Variation across the interface sites can facilitate orthologous species ACE2 receptor usage, since as few as seven interface sites are needed for entry. The prefusion structure of the S glycoprotein has three major antigenic domains, receptor binding domain (RBD), N terminal Domain (NTD), and S2. Epitopes on SARS-CoV-1 RBD have been identified as targets for neutralizing antibodies. Analyzing the variations and conserved regions in the S protein of Group 2B SARS-like CoVs, shows conserved sites on the S2 region that could be targeted in broad-based therapeutics against multiple CoVs.
Dr. Baric stressed that there is a large reservoir of SARS-like and MERS-like CoVs poised for emergence in humans. Two priorities are immediate vaccine candidates specific for SARS-CoV-2 and development of broad-based vaccines protective against antigenically distinct CoVs destined to emerge in the future. Key priorities for the development of a SARS-CoV-2 vaccine include characterization the SARS-CoV-2 neutralizing epitope map, identification of broadly cross-reactive neutralizing epitopes, identification of putative enhancing epitopes that might potentiate disease in vivo, identification of key T cell epitopes across outbred populations, and determination of correlates of protective immunity.

5.2 Preserving Neutralization Sensitive Epitopes on Spike Proteins

Barney Graham, MD PhD, Deputy Director of the NIH Vaccine Research Center presented data on the immunogenicity and neutralizing efficacy of truncated spike (S) antigens, with a focus on SARS-CoV-2. Class I fusion proteins (such as S protein) are common among enveloped viruses including RSV, parainfluenza viruses, and coronaviruses and have been successfully stabilized in their pre-fusion conformations. This approach has been shown to preserve neutralization-sensitive epitopes, avoid antibodies that are non-neutralizing, and improve expression in transfected cells, thus aiding in manufacturing and immunogenicity of gene-based vectors. The S proteins of SARS-CoV-1 and MERS-CoV have both been successfully stabilized by introducing two proline residues to the top of the central helix, preventing heptad assembly and stabilizing the S2 region and the entire S protein as a result (Figure) [46].

Figure: 2P mutation stabilizes MERS and SARS CoV S; improves expression, prefusion structure, and immunogenicity



The SARS-CoV-2 S protein structure was solved shortly after its emergence and shows similar structure and mobility as the SARS-CoV-1 S [47]. The timing from first knowledge of SARS-CoV-2 to the beginning of the Phase 1 study was a remarkable sixty-five days. The advantages of mRNA vaccines include ability to create a highly precise type of protein to elicit the correct antibodies, to elicit T cell responses that are Th1 predominant, and the rapidity of manufacturing. Of course, disadvantages include the novel nature of both mRNA and DNA vaccines without any licensed vaccine with either technology to date and lack of experience for mass production. Therefore, multiple platforms for SARS-CoV-2 are under development that mitigate against some of the potential disadvantages of nucleic acid vaccines.

6. Effects of Adjuvants on Immune Response and Implications for COVID-19 Vaccines

Although mRNA and DNA vaccines elicit T cell responses without adjuvants, adjuvants may be important for subunit and whole cell inactivated vaccines to increase their immunogenicity and drive an immune response that could limit the risk of disease enhancement. Multiple SARS-CoV-2 vaccines are in development including vectored vaccines, whole cell inactivated vaccines, and recombinant protein vaccines. The experts discussed how the choice of adjuvants will be important for both efficacy and safety with these platforms.

Dr. Arnaud Didierlaurent from the Centre of Vaccinology at the University of Geneva presented background on the effects of different adjuvants on animal and human immune responses. Several adjuvants are now being used in commercial vaccines or are in clinical development [48]. Oil-in-water emulsions such as MF59 or AS03 have been shown to increase the breadth of the antibody repertoire, binding affinity and affinity maturation when compared to unadjuvanted vaccines [49, 50] In human studies with influenza vaccines, H5N1 vaccine adjuvanted with MF59 (squalene-based emulsion) increased the levels of H5-specific antibody for subclasses IgG1 and IgG3 and the binding to FcγR2 but not to FcγR3 when compared to alum adjuvanted vaccines. This demonstrates that the use of an adjuvant can skew the functionality profile of antigen-specific antibodies, with the potential to activate different innate effectors based on their FcγR expression [51]. Use of squalene-based emulsion vaccines for influenza have also been shown to increase CD4+ T cell response frequencies and cross-reactivity. Even if pre-existing cross-reactive antibodies are present prior to immunization, such adjuvants could activate naïve B cells and promote the adaptability of memory B cells [52-55].

In addition to antibodies, adjuvants can promote cellular responses. Human malaria challenge studies provided early evidence that the choice of adjuvants(combined with the malaria antigen RTS,S) was critical in achieving optimal protection and highlighted the importance of cellular response [56]. More recently, studies with Hepatitis B Surface Antigen (HBs) vaccine adjuvanted with AS01, AS03, AS04 or Alum showed that vaccines formulated with AS01 and AS03 induced the highest antibody levels while AS01 promoted best HBs-specific CD4 T cell response [57]. These differences were associated with the magnitude of the initial inflammatory response triggered by the different adjuvanted formulations [57, 58]. Interestingly, although the level of CD4 T cell response was lower in the alum group compared to the AS01 group, both adjuvants led to similar memory subset profiles and cytokine production profiles (polyfunctionality) and neither induced Th2 cytokines nor a CD8 induced response upon peptide restimulation. This indicates that use of alum may not necessarily lead to Th2 skewing in humans. Recently a number of systems biology studies have revealed that specific early signatures (e.g., interferon-dependent pathways) induced by adjuvanted vaccines are often associated with protective responses [59] but the impact of these early signals on functional features of antibodies and the quality of T cell response is not well established yet.

Although adjuvant selection is best performed in early clinical studies, animal models could be useful is determining immune profile of adjuvanted vaccines. NHP models are well-established to assess immune responses to vaccination and elicit immune responses in closer parallel to humans than mice. For example, in non-human primates, adjuvant choice affects antibody half-life, antibody glycosylation and antibody binding to FcγRs, indicating effects on both antibody quality and function, like what is observed in humans [60]. When adeno-based vectored vaccines are given to humans or NHPs, both groups develop similar antibody function profiles. Additionally, NHPs and humans tend to show similarities in terms of “ranking” of adjuvants and innate immune pathways triggered by adjuvants. Overall, NHPs could be utilized to evaluate COVID-19 vaccine candidates with and without adjuvants and guide in the selection of vaccines that elicit desired attributes that could reduce the risk of vaccine-mediated enhanced disease.

Given the unprecedented demand for an effective vaccine, the use of adjuvants may be critical for subunit vaccines in providing antigen-dose sparing, increased immunogenicity, breadth and duration of response, potentially eliciting cross-protection against new CoV strains and minimizing the risk of enhanced disease.

7. Consensus Considerations on the Assessment of the Risk of Disease Enhancement with COVID-19 Vaccines

Following the presentations, attendees participated in discussion of the suggested consensus statements and all attendees were asked to comment on the draft statements available online. These comments were

reviewed and discussed again on the second day of the meeting and resulted in the summary consensus statement that follows.

Murine models for assessment of vaccine-related disease enhancement

- SARS-CoV-2 has a low affinity for murine ACE2 receptor and murine models will require the use of hACE2 transgenic mice, preferably with a 'knock-in' approach. Preliminary data indicate the possibility of infecting hACE2 transgenic mice with demonstration of viral replication and mild lung lesions. Mouse adaptation of SARS-CoV-2, as done with SARS-CoV-1, will likely be required to obtain a virus that causes more severe disease in mice. Models that develop acute lung injury with some lethality and that mimic the human condition will be important for evaluating vaccine safety.
- Previous studies from SARS-CoV-1 and MERS-CoV indicated that some vaccines, especially those using whole inactivated virus, could enhance the disease induced in mice challenged with SARS-CoV-1 or MERS-CoV. The lung lesions were highly inflammatory, with a dominance of eosinophil infiltration and occurred in animals despite presence of a neutralizing antibody response and reduced challenge virus replication in the lungs. Such studies have not yet been completed for SARS-CoV2.
- In mice, this immunopathology was considered a consequence of a dominant Th2 type response to the vaccine antigens. It was not seen after including adjuvants (e.g. CpG) in the vaccine or other vaccine formulations known to drive immune responses towards Th1. The timing of challenge after vaccination may be critical. It would be of major interest to explore the outcome following challenge at later timepoints when antibodies are significantly decaying.
- One should be aware of the potential confounding effect of cell-culture excipients in the vaccine and challenge strain material. It is known that impurities such as fetal calf serum in the preclinical vaccine preparation may induce eosinophil influx in any mouse model if the challenge strain also contains the same excipients.
- In these models, characterization of the immune response to the candidate vaccine (e.g., IgG isotypes, Th2 markers) may have some predictive value.
- Other small animal models which can be infected by SARS-CoV-2 can be considered (e.g. ferret, hamster). Development of small animal models of severe disease will also inform studies of vaccine-enhanced disease.

Non-human primate models for assessment of vaccine-mediated enhanced disease

- Non-human primates (NHP) are of primary interest in view of their ACE2 homology with hACE2. Preliminary studies indicate the possibility of inducing some COVID-19 lung pathological features after infection, without clinical signs, in Rhesus macaques. African Green monkeys may be more susceptible to COVID-19, but the model suffers from some limitations (e.g. access, genetic polymorphism).
- Previous studies with SARS candidate vaccines have suggested a risk of enhanced pathology in NHPs after viral challenge. Eosinophilic infiltrates were not prominent. The mechanism is still incompletely defined but there is evidence for a role of non-neutralizing antibodies. Non- or incompletely neutralizing antibodies may contribute to:
  - the formation of pathogenic immune complexes and
  - Fc-mediated viral capture by monocytes/macrophages that may favor excessive T-cell activation and inflammation.
- Enhanced pathology was seen following passive transfer of IgG from immunized NHPs

General considerations on animal models

- Although existing animal models of COVID-19 imperfectly reproduce the human disease, they appear useful for assessing the risk of disease enhancement. Vaccine responses are closer to human responses in NHPs than in mice. Therefore, it is likely that data obtained from NHP studies are more significant. However, there is an urgent need to standardize the NHP model (read-out of disease enhancement, timing of challenge, age) and to include appropriate controls (i.e., a vaccine that induces enhanced pathology and disease) and a sufficient number of animals to be confident of findings in outbred species. It is important to control for potential co-infection, including with other coronaviruses, in all non-SPF models.
- Potential markers of safety in these animal models could include:
  - the relative levels of neutralizing vs non-neutralizing antibodies,
  - antibody affinity,
  - T-cell response profile,
  - quantitative virology in the upper and lower respiratory tract
  - characterization of lung histopathology with immunohistochemistry for viral antigen and immune cell markers.
- Passive transfer in NHPs of human antibodies generated during Phase 1 trials, followed by viral challenge could be considered to assess the risk of disease enhancement.
- Challenge of immunized animals with a closely related heterologous CoV strains may assess the risk of enhancement during future outbreaks.
- In case of disease enhancement, in-depth studies in animal models may give some indications on the mechanism of immunopathology. They can inform human trial designers on the critical immunological risk markers to be monitored in Phase 1 trials.
- Based on previous experience with SARS and other viral diseases, it may be useful to evaluate the risk of disease enhancement for COVID-19 vaccines (particularly those including whole virions or N protein) in an established NHP model before advanced clinical development.

During the Vaccine Design session, the group of Experts suggested that consideration should be given to the following:

- Caution should be observed when developing vaccines to avoid inducing predominant Th2 responses and non-neutralizing antibodies.
- Vaccines inducing strong neutralizing antibodies, predominant Th1 responses and balanced CD4/CD8 and polyfunctional T cell responses are less likely to induce immunopathology.
- Given what will be the unprecedented demand for an effective vaccine, the use of adjuvants may be critical for sub-unit vaccines in providing increased immunogenicity, breadth of response, dose sparing, duration of response, potentially cross-protection against new CoV strains, and possibly minimize the risk of enhanced disease. Preference should be given to Th1-driving adjuvants with an established safety profile in humans.
- Understanding of the role of cross-reacting antibodies from prior coronavirus infections may have on natural disease caused by SARS-CoV-2 or influence the risk of enhanced disease following vaccination may inform vaccine design.
- Data are needed on whether antibody waning could increase the risk of enhanced disease on exposure to virus in the long term.

It was the opinion of the Experts that animal data to support clinical development could address:

- Post-vaccination (neutralizing) antibody responses, and T cell analysis to demonstrate a Th1 response.
- Post-vaccination challenge data from NHPs with careful evaluation for immunopathology and quantitative virology in the animals.
- Small animal data may also provide important supporting evidence of safety, and hamster, ferret and mouse models are likely to be available for developers.
- Where possible, immunopathology experiments with a positive control (e.g., formalin inactivated alum-adjuvanted SARS-CoV-1 or SARS-CoV-2 vaccine) and a mock-immunized negative control will provide best guidance. It was felt that it will be important to establish broadly accepted endpoints and scoring systems to allow comparison of various vaccine candidates. WHO is working on this issue.
- For vaccine constructs likely to induce a predominant Th2 response, the group felt that animal studies should be considered before entering human Phase 1 trials in more than one animal species including NHPs where possible. It was noted that the absence of a Th2 response does not eliminate the risk of enhanced disease.
- For vaccine constructs which are already known to induce neutralizing antibody and Th1 responses, it was the consensus of the group that while Phase 1 studies are cautiously proceeding with careful review of safety data, animal studies run in parallel could provide useful information for the further clinical development
- Suggestive data in animal models should not by default prevent clinical development of vaccine candidates; potential risk should be thoroughly evaluated by developers and regulators on a vaccine product-specific basis.

Regarding Phase 1 clinical trials, it was the opinion of the Experts that:

- Since not all studies that have begun or are about to begin will prescreen to determine preimmunization serostatus of participants, although this shall be determined retrospectively, appropriate baseline blood specimens should be obtained and stored. Because the virus is spreading rapidly, such specimens will allow assessment of the immune response in both seronegative and seropositive persons as both are likely to be vaccinated.
- Level of neutralizing antibodies and determination of the relative ratio of binding to neutralizing antibodies will be important to assess the potential risk of enhanced disease. Also, detection of initial priming that includes CD8 T cells and/or a CD4 Th1 biased response is likely to mitigate the risk of disease enhancement. Determination of memory responses will be useful, particularly if SARS-CoV-2 continues to circulate.
- Consideration should be given to the use of post-vaccination sera from vaccinees which could be used for antibody transfer studies in animals to look for enhanced disease and for evidence of cross-protection against other coronaviruses.
- Monitoring for enhanced disease in immunized participants may require longer follow-up than is usual in Phase 1 trials but need not delay Phase 2 trials.
- Investigators on the call requested frequent updating with both preclinical and evolving clinical data that are being developed by the different academic and industrial developers to help in decision-making about the various vaccine clinical trials. Creation of a central information hub was encouraged for this purpose.
- Participants on the call expressed the need for standardization of protocols, data collection forms, critical assays (including reagents) and biobanking of samples from initial clinical trials to allow future re-assay once standards are agreed to and enable comparison of results across trials

Concluding remarks

- The group of Experts considers that the demonstration of some disease enhancement with any candidate vaccine after viral challenge in animal models should not necessarily represent a no-go signal for deciding whether to progress into early trials in clinical development of a COVID-19 vaccine.
- Continuous monitoring of this risk during clinical trials in an epidemic context will be needed.
- Each observed effect should be discussed by the developers with their regulators who will ultimately define the actual requirements for clinical studies.

The considerations in this document should be interpreted as general scientific remarks based on current knowledge to inform a research agenda that could be beneficial for vaccines in development. These considerations are not of a regulatory nature and cannot in any sense replace the need for proper regulatory consultations on the requirements for vaccines clinical trials. Vaccine developers are therefore encouraged to seek individual scientific advice from regulatory authorities.

Declaration of Competing Interest

RB has collaborations with VaxArt, Takeda, Moderna, Eli Lily, and Pfizer. SB is a consultant for GSK on matters unrelated to the topic of this manuscript. CD is a consultant to Medicago on their vaccine programs; her husband owns stock in Dynavax Technologies Corporation. BSG is a named inventor on patent applications related to coronavirus vaccines and monoclonal antibodies. AJP is Chair of UK Dept. Health and Social Care's (DHSC) Joint Committee on Vaccination & Immunisation (JCVI) and is a member of the WHO's SAGE. AJP is an NIHR Senior Investigator. PL, DA, SRA, RTC, AMD, SDM, DM, SP, PAP, CQ, and KS declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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All authors attest they meet the ICMJE criteria for authorship

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