COVAX

Window 1

Information Fact Pack

22 March 2021

COVAX

Information Fact Pack

COVAX

Candidate	Ad26COV2.S
Manufacturer	Janssen Pharmaceutica NV
Headquarter location	Belgium

Technical information

Technology platform	Non-replicating viral vector
Antigen / adjuvant	Recombinant human Adenoviral vector serotype 26 (Ad26) expressing the spike glycoprotein from SARS-CoV2 No adjuvant
Vaccine presentation	Multi-dose vial (5 dose, 0.5mL)
Expected indication	For the prevention of COVID-19 in adults aged 18 years and older
Expected schedule	1-dose schedule
Route and site of administration	Intramuscular (IM) injection
Stability and storage	2 years at -20°C 3 months at 2-8°C
Labelling / Packaging	Janssen will provide the doses in a generic/standardized packaged and labeled form suitable for usage. Janssen cannot accommodate any country-specific, non-generic or non-standardized packaging or labelling requests. The proposed packaging and labels are being reviewed with the World Health Organization (WHO).
Efficacy data	Primary efficacy analysis of Phase III data was based on 39,321 participants accruing 259 symptomatic cases of COVID-19 in the Phase III ENSEMBLE trial of the single-dose vaccine. Primary analysis suggests the vaccine has an overall efficacy of 66% [95% CI 55.0 to 74.8] in preventing moderate to severe COVID-19, 28 days after vaccination (72% in the United States, 68% in Brazil, and 64% in South Africa). Predominant strains among those sequenced were the Wuhan-H1 variant D614G in the U.S. (96.4% of sequenced cases), 20H/501Y.V2 variant (B.1.351) in South Africa (94.5% of sequenced cases), a variant of the P.2 lineage in Brazil (69.4% of sequenced cases), and the Wuhan-H1 variant D614G (39.6%) in remaining cases. As of February 12, 2021, no cases had been identified as B.1.1.7 or P1 lineages. Protection was generally consistent across race, age groups, including adults over 60 years of age (n=>13,000), and across variants and regions studied. The vaccine was 85% [95% CI: 54.2 to 96.9) effective in preventing severe disease, 28 days post-vaccination. The vaccine demonstrated complete protection against COVID-related hospitalization and death, 28 days post-vaccination.
Safety / reactogenicity data	Safety analysis through the January 22, 2021 data cutoff included 43,783 participants ≥18 years of age with 2-month median follow-up. The analysis supported a favorable safety profile with no specific safety concerns and mild-to-moderate reactogenicity.

Clinical and regulatory timelines

Clinical development overview	Phase I/II sites Belgium and United States (Phase I/II) – Official registration: NCT04436276 Japan (Phase I) – Official registration: NCT04509947 Phase III sites Argentina, Brazil, Chile, Colombia, Mexico, Peru, Philippines, South Africa, Ukraine, United States Official registration for ENSEMBLE trial: NCT04505722
Regulatory strategy	FDA: Emergency Use Authorization (EUA) granted on February 27, 2021. EMA: Conditional Marketing Authorization (CMA) granted on March 11, 2021. WHO: Emergency Use Listing (EUL) granted on March 12, 2021.

Indicative COVAX Facility deal terms

Total supply to COVAX Facility	200 million doses, with a potential additional 300 million doses subject to agreement between GAVI and Janssen
Estimated date of first delivery	Janssen aims to deliver from Q3 2021
Purchase Price (price band per tier)[1]	UMICs – Band B: >6-13 USD / dose HICs – Band B: >6-13 USD / dose
Upfront payment to be made by COVAX Facility[2]	Band I: 5-15% of Purchase Price
Estimated date of second opt-out window	Q2 2021
Supply conditions	As a condition to Janssen making available vaccine doses for supply, each recipient COVAX Participant is required to (amongst other matters): - adopt a No Fault Compensation System as further described below; - accept Janssen's standard indemnity provision (non-negotiable, and further described below) as well as Janssen's purchase terms (including standard delivery and ordering requirements), by entering an agreement with Janssen to such effect; - pay the full price for the vaccine doses to Janssen (directly or through UNICEF/PAHO if procuring through the Procurement Agencies); and - have in place the processes and procedures necessary to ensure that the maintenance, distribution, storage, transport, administration and management of the vaccine doses, along with any related follow-on care, are in accordance with current good distribution practices, applicable specifications, and Janssen's instructions for storage and distribution (including cold chain requirements), all of which will be made available by Janssen in due course.

[1] Standardized price bands used for comparison across all vaccines within the COVAX Facility Portfolio. Band A: <6 USD/dose; Band B: >6-13 USD/dose; Band C: >13-21.1 USD/dose; Band D: >21.1 USD/dose.

[2] Standardized upfront payment bands used for comparison across all vaccines within the COVAX Facility Portfolio: Band I: 5-15%, Band II: 15-25%, Band III: >25%.

No Fault Compensation System

As a condition to supply, each recipient COVAX Participant is required to have in place and maintain a No Fault Compensation System in accordance with the following minimum requirements. The No Fault Compensation System must have been adopted/established prior to Janssen making available for supply any vaccine dose to such recipient COVAX Participant, and must be maintained in full force and effect after the vaccine doses have been supplied.

Janssen states that these requirements are standard Janssen requirements applicable to Janssen customers generally, including those outside of the COVAX Facility. For those COVAX Participants who have a direct, bilateral advance purchase agreement with Janssen for the Janssen COVID-19 Vaccine which agreement specifies a No Fault Compensation System, that system, if applicable to doses of the Janssen vaccine procured via COVAX, will satisfy this requirement.

1. No-Fault Compensation System

a. Victims should only be required to demonstrate a causal link between the vaccine and the relevant damages, without the need to prove negligence, fault or product defect.
b. Victims should be required to demonstrate causation by a preponderance of the evidence (or a similar evidentiary standard).

2. Administrative structure

a. System should be administered by a public administrative body.
b. System should include an adequate public funding mechanism but additional financing sources can be added.

3. Governance structure

a. Administrative bodies should include representation from diverse stakeholders.
b. Decision making panels should be composed of experts with clearly defined requirements (medical, legal).

4. Covered vaccines

a. Systems should cover injuries resulting from Covid-19 vaccines. Systems may cover injuries from other classes of vaccines as well, but the funding needs to be separate for Covid-19 vaccines.
b. Applicants can be anyone who has been administered a Covid-19 vaccine in the Territory.

5. Covered damages

a. System should cover a reasonably broad class of damages, including death, injury, disability, pain and suffering, and other forms of economic and noneconomic loss resulting from the injury.
b. Minor injury and resulting damages should not be covered.

6. Compensation

a. The level of compensation offered by the System, as supplemented by other governmental arrangements (e.g., social security programs), should be sufficient to provide long-term relief to victims.
b. Compensation could be tariff-based, consistent with the level of compensation as per 6a.

7. Accessible and Efficient Procedures

a. System should use simple and easily available intake forms.
b. Bringing claims should not require legal assistance.
c. Bringing claims should be free of charge.
d. The review and decision-making process should be well-defined and easily understood by victims.
e. System should have reasonably efficient timelines for processing claims and rendering decisions.
f. System should allow victims to appeal decisions within the compensation system and finally through a court system (adequate legal remedies), with such appeal being directed against the compensation system (not against the manufacturer or any other party).
g. The Participating Government should implement strategies to ensure broad public awareness of their compensation system.

h. System needs to be properly resourced (personnel, funding, organization) and have the proper infrastructure, in particular IT, to handle the case load.

8. Transparency

a. System should include formal, well-defined transparency measures, such as mandatory annual reports and/or requirements to regularly provide public access to system information (e.g., claims received, claims excepted, and compensation amounts).

Janssen's standard indemnity provision

1. Participating Government shall indemnify and hold harmless Janssen, its affiliates, sub-contractors and sub-licensees, all of its partners and third party contractors involved in or otherwise contracted for the design, research, development (including pre-clinical and clinical testing), manufacturing (including contract manufacturers), packaging and labelling (including warnings), procurement, storage, distribution and deployment of the Vaccine Candidate, as well as its and their respective officers, directors, employees and other agents and representatives (together, the "**Indemnified Persons**") from any and all (i) losses, claims (including, without limitation, claims for personal injury or death), actions, liabilities, damages, judgments and awards, (ii) costs and expenses pertaining to or resulting from the defense, resolution (including settlement) or processing of any such losses, claims, actions, liabilities, damages, judgments or awards (including attorneys' and other professional advisors' fees and expenses (including taxation)), and (iii) procedural costs (including penalties, interest, fines and taxes on court ordered payments) ((i) to (iii) together, the "**Losses**") suffered or incurred by, or against, the Indemnified Persons in connection with any demands, claims, actions or proceedings of any kind:
   a. involving, relating to, or arising out of or in connection with the Vaccine Candidate (including, and regardless of whether the alleged cause of the damage originates from, the design, research, development, testing, manufacture, labelling, packaging, sale, procurement, delivery, deployment, distribution, storage, administration, effects and/or use of the Vaccine Candidate);
   b. brought or initiated by or on behalf of:
      i. the Participating Government or any state, provincial, municipal, local or regional governments or competent public authorities within such Participating Country (the "Territory"), or any of its or their respective agencies, departments, ministries, bodies, governments (local, regional or federal) or other public authorities of any kind; or
      ii. a Vaccinated Individual whose Residence is in the Territory (irrespective of the nationality, citizenship or country of origin or incorporation of such Vaccinated Individual); or
      iii. a Vaccinated Individual who has been administered the Vaccine Candidate in the Territory (even if such Vaccinated Individual's Residence is not in the Territory); or
      iv. any other person in the courts of competent jurisdiction of the Territory, including within any state, province, municipality or locality thereof.

2. The indemnification right under paragraph 1 will not be available to the Indemnified Persons to the extent that their Losses result directly from the Adjudicated Willful Misconduct or Adjudicated Failure to comply with Good Manufacturing Practices ("GMP") of such Indemnified Persons, where:
   a. "**Willful Misconduct**" shall mean an act or omission that is taken (i) with intentional disregard of a known risk in the manufacture of the Vaccine Candidate that is so great as to make it highly probable that the harm will outweigh the benefit, (ii) without legal or factual justification, and (iii) with the intent of achieving a wrongful purpose (it being understood, however, that any action consistent with rules or guidance set out by Participating Government or any other government (be it state, provincial, municipal, local or regional) in the Territory, or any public agency, body or other public or regulatory authority in the Territory, and any action, test or results disclosed to a regulatory authority as a part of receiving regulatory approval for the Vaccine Candidate in the Territory shall not be considered to be Willful Misconduct);
   b. "**Failure to comply with GMP**" shall mean a failure of compliance with the GMP rules directly causing death or serious physical injury or illness of a Vaccinated Individual.
   c. "**Adjudicated"** shall mean a final determination by a court of competent jurisdiction for which the time for filing an appeal has expired and all appeals have been exhausted.

3. If any person makes a claim or initiates a demand, claim, action or proceeding (or notifies in writing an intention to do so) against an Indemnified Person which, in the reasonable opinion of Janssen is considered likely to result in indemnification under paragraph 1 above (a "**Claim**"), Janssen shall:

a. as soon as reasonably practicable, give written notice of the Claim to the Participating Government, specifying the nature of the Claim in reasonable detail (insofar as available), provided that the failure to promptly provide such notice shall not relieve the Participating Government of its indemnification obligations under paragraph 1; and

b. in Janssen's sole discretion:

   i. either take such actions as it may consider reasonable and appropriate to avoid, dispute, compromise or defend the Claim (with all related costs, fees and expenses, as well as Losses, to be paid by the Participating Government), provided that Janssen may settle the Claim only with the prior consent of the Participating Government (such consent not to be unreasonably withheld, conditioned or delayed); or

   ii. require the Participating Government to assume (with its own counsel and at its own costs) sole control of the defence or settlement of the Claim and substitute, where possible under applicable law, the Participating Government as the defendant; provided that in such case:

      A. the Participating Government shall reasonably take into consideration the interests (commercial, corporate, reputational or other) of Janssen and shall not conclude any agreement or make any compromise or settlement with any person in relation to such Claim without the prior written consent of Janssen (such consent not to be unreasonably conditioned, withheld or delayed); and

      B. Janssen shall have the right, but not the obligation, to participate in the defence or settlement of the Claim and to retain its own counsel in connection with such Claim; and

      C. Janssen shall provide assistance and information reasonably required by the Participating Government in the defense of the Claim (at the expense of the Participating Government), provided that (a) any information reasonably considered by Janssen as confidential or proprietary information shall be provided by it only if and when satisfactory confidentiality arrangements are put in place, and (b) under no circumstances shall Janssen provide any information (including trade secrets) which it reasonably believes would cause material harm to it or other Indemnified Persons if disclosed.

4. Participating Government's obligation to indemnify the Indemnified Persons for Claims under paragraph 1 is not subject to a financial limitation or maximum, nor is it limited by the number of indemnifiable Claims brought against the Indemnified Persons.

5. It is the intention of the Participating Government to constitute Janssen as a trustee for and agent of the Indemnified Persons that are not party to this Agreement of the covenants of the Participating Government contained in paragraphs 1 to 4 above and Participating Government agrees that Janssen may enforce the indemnity covenants of the Participating Government contained in paragraphs 1 to 4 above for and on behalf of each applicable Indemnified Person and, in such event, the Participating Government will not, in any proceeding to enforce the indemnity by or on behalf of the applicable Indemnified Persons, assert any defense thereto based on the absence of authority or consideration or privity of contract and irrevocably waives the benefit of any such defense.

6. The Parties acknowledge and agree that the provisions of paragraphs 1 to 5 are reasonable and necessary to protect the legitimate interest of the Indemnified Persons. However, if any provision in paragraphs 1 to 5 is held to be illegal, invalid or unenforceable, in whole or in part, under any applicable law, then such provision shall not be nullified but the Parties shall be deemed to have agreed to such provision that conforms with the limitations imposed by applicable law and that is as close as possible to the original intention of the Parties and has the same or as similar as possible economic effect, and such provision shall be automatically reformed accordingly.

Defined terms

"**Residence**" means the place of permanent home or principal establishment;

"**Vaccine Candidate**" means Janssen's investigational COVID-19 vaccine based on its proprietary recombinant, replication defective adenovirus 26 (Ad26) vector, supplied to the Participating Government under the Advance Purchase Agreement;

"**Vaccinated Individual**" means any individual who has been administered the Vaccine Candidate (or, as the case may be, any individual, group, entity or organization purporting to represent, act on behalf or, recover for or in respect of, or seek damages with respect to, any such individual or group of such individuals)

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Appendix: Publications and Press Releases

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Appendix

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Candidate	Ad26COV2.S
Manufacturer	Janssen Pharmaceutica NV

List of scientific publications

Journal	Author	Date of publication
Nature	Mercado *et al.*	July, 2020
Nature Medicine	Tostanoski *et al.*	September, 2020
NPJ Vaccines	Bos *et al.*	September, 2020
The New England Journal of Medicine	Sadoff *et al.*	January, 2021

List of press releases

Title	Date of release
Johnson & Johnson Launches Multi-Pronged Response to Coronavirus Global Public Health Threat	January 29, 2020
Johnson & Johnson Announces Collaboration with U.S. Department of Health & Human Services to Accelerate Development of a Potential Novel Coronavirus Vaccine	February 11, 2020
Johnson & Johnson Announces Collaboration with the Beth Israel Deaconess Medical Center to Accelerate COVID-19 Vaccine Development	March 13, 2020
Johnson & Johnson Announces a Lead Vaccine Candidate for COVID-19; Landmark New Partnership with U.S. Department of Health & Human Services; and Commitment to Supply One Billion Vaccines Worldwide for Emergency Pandemic Use	March 30, 2020
Johnson & Johnson Announces Collaboration to Expand Manufacturing Capabilities For its COVID-19 Vaccine Candidate in Support of the Company's Goal to Supply More Than One Billion Vaccine Doses Globally	April 23, 2020
Johnson & Johnson Announces Acceleration of its COVID-19 Vaccine Candidate; Phase 1/2a Clinical Trial to Begin in Second Half of July	June 10, 2020
Single Dose of Johnson & Johnson COVID-19 Vaccine Candidate Demonstrates Robust Protection in Pre-clinical Studies	July 30, 2020
Johnson & Johnson Announces Collaboration in Principle with the United Kingdom on Additional Phase 3 Study and Agreement to Supply its COVID-19 Vaccine Candidate	August 14, 2020
Johnson & Johnson Announces that Janssen's COVID-19 Investigational Vaccine Candidate Prevents Severe Clinical Disease in Pre-clinical Studies	September 3, 2020
Johnson & Johnson Initiates Pivotal Global Phase 3 Clinical Trial of Janssen's COVID-19 Vaccine Candidate	September 23, 2020
Johnson & Johnson Posts Interim Results from Phase 1/2a Clinical Trial of its Janssen COVID-19 Vaccine Candidate	September 25, 2020 (updated on October 4, 2020)
Johnson & Johnson Temporarily Pauses All Dosing in Our Janssen COVID-19 Vaccine Candidate Clinical Trials	October 13, 2020
Johnson & Johnson Prepares to Resume Phase 3 ENSEMBLE Trial of its Janssen COVID-19 Vaccine Candidate in the U.S.	October 23, 2020

Johnson & Johnson and U.S. Department of Health & Human Services Expand Agreement to Support Next Phase of COVID-19 Vaccine Candidate Research and Development	November 14, 2020
Johnson & Johnson Initiates Second Global Phase 3 Clinical Trial of its Janssen COVID-19 Vaccine Candidate	November 15, 2020
Johnson & Johnson Announces Initiation of Rolling Submission for its Single-dose Janssen COVID-19 Vaccine Candidate with the European Medicines Agency	December 1, 2020
Johnson & Johnson Announces Its First Phase 3 COVID-19 Vaccine Trial ENSEMBLE is Fully Enrolled	December 17, 2020
Johnson & Johnson Announces Agreement in Principle with Gavi to Supply Janssen's COVID-19 Vaccine Candidate to Lower-Income Countries in 2021	December 18, 2020
Johnson & Johnson COVID-19 Vaccine Candidate Interim Phase 1/2a Data Published in New England Journal of Medicine	January 13, 2021
Johnson & Johnson Announces Single-Shot Janssen COVID-19 Vaccine Candidate Met Primary Endpoints in Interim Analysis of its Phase 3 ENSEMBLE Trial	January 29, 2021
Johnson & Johnson Announces Submission of Application to the U.S. FDA for Emergency Use Authorization of its Investigational Single-Shot Janssen COVID-19 Vaccine Candidate	February 04, 2021
Johnson & Johnson Announces Submission of European Conditional Marketing Authorisation Application to the EMA for its Investigational Single-Shot Janssen COVID-19 Vaccine Candidate	February 16, 2021
Johnson & Johnson Announces Submission to World Health Organization for Emergency Use Listing of Investigational Single-Shot Janssen COVID-19 Vaccine Candidate	February 19, 2021
Johnson & Johnson COVID-19 Vaccine Authorized by U.S. FDA For Emergency Use - First Single-Shot Vaccine in Fight Against Global Pandemic	February 27, 2021
Johnson & Johnson Single-Shot COVID-19 Vaccine Granted Conditional Marketing Authorization by European Commission	March 11, 2021
Johnson & Johnson Single-Shot COVID-19 Vaccine Granted Emergency Use Listing by the World Health Organization	March 12, 2021
Statement on the Interim SAGE Recommendation Supporting the Use of the Johnson & Johnson COVID-19 Vaccine	March 17, 2021

Article

Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques

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Noe B. Mercado[1,10], Roland Zahn[2,10], Frank Wegmann[2,10], Carolin Loos[3,4,10], Abishek Chandrashekar[1,10], Jingyou Yu[1,10], Jinyan Liu[1,10], Lauren Peter[1,10], Katherine McMahan[1,10], Lisa H. Tostanoski[1,10], Xuan He[1,10], David R. Martinez[5,10], Lucy Rutten[2], Rinke Bos[2], Danielle van Manen[2], Jort Vellinga[2], Jerome Custers[2], Johannes P. Langedijk[2], Ted Kwaks[2], Mark J. G. Bakkers[2], David Zuijdgeest[2], Sietske K. Rosendahl Huber[2], Caroline Atyeo[3,6], Stephanie Fischinger[3,6], John S. Burke[3], Jared Feldman[3,6], Blake M. Hauser[3,6], Timothy M. Caradonna[3,6], Esther A. Bondzie[1], Gabriel Dagotto[1,6], Makda S. Gebre[1,6], Emily Hoffman[1], Catherine Jacob-Dolan[1,6], Marinela Kirilova[1], Zhenfeng Li[1], Zijin Lin[1], Shant H. Mahrokhian[1], Lori F. Maxfield[1], Felix Nampanya[1], Ramya Nityanandam[1], Joseph P. Nkolola[1], Shivani Patel[1], John D. Ventura[1], Kaylee Verrington[1], Huahua Wan[1], Laurent Pessaint[7], Alex Van Ry[7], Kelvin Blade[7], Amanda Strasbaugh[7], Mehtap Cabus[7], Renita Brown[7], Anthony Cook[7], Serge Zouantchangadou[7], Elyse Teow[7], Hanne Andersen[7], Mark G. Lewis[7], Yongfei Cai[8], Bing Chen[8,9], Aaron G. Schmidt[3,6,9], R. Keith Reeves[1], Ralph S. Baric[5], Douglas A. Lauffenburger[4], Galit Alter[3,9], Paul Stoffels[2], Mathai Mammen[2], Johan Van Hoof[2], Hanneke Schuitemaker[2,11] & Dan H. Barouch[1,3,6,9,11]✉

A safe and effective vaccine for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may be required to end the coronavirus disease 2019 (COVID-19) pandemic[1–8]. For global deployment and pandemic control, a vaccine that requires only a single immunization would be optimal. Here we show the immunogenicity and protective efficacy of a single dose of adenovirus serotype 26 (Ad26) vector-based vaccines expressing the SARS-CoV-2 spike (S) protein in non-human primates. Fifty-two rhesus macaques (*Macaca mulatta*) were immunized with Ad26 vectors that encoded S variants or sham control, and then challenged with SARS-CoV-2 by the intranasal and intratracheal routes[9,10]. The optimal Ad26 vaccine induced robust neutralizing antibody responses and provided complete or near-complete protection in bronchoalveolar lavage and nasal swabs after SARS-CoV-2 challenge. Titres of vaccine-elicited neutralizing antibodies correlated with protective efficacy, suggesting an immune correlate of protection. These data demonstrate robust single-shot vaccine protection against SARS-CoV-2 in non-human primates. The optimal Ad26 vector-based vaccine for SARS-CoV-2, termed Ad26.COV2.S, is currently being evaluated in clinical trials.

The rapid expansion of the COVID-19 pandemic has made the development of a SARS-CoV-2 vaccine a global health priority. Ad26 vectors[11] that encode viral antigens have been shown to induce robust humoral and cellular immune responses to various pathogens in both non-human primates and humans. In this study, we developed a series of Ad26 vectors that encode different variants of the SARS-CoV-2 spike (S) protein, and evaluated their immunogenicity and protective efficacy against SARS-CoV-2 challenge in rhesus macaques.

Generation and immunogenicity of Ad26 vectors

We produced seven Ad26 vectors that expressed SARS-CoV-2 S variants that reflected different leader sequences, antigen forms and stabilization mutations: (i) tissue plasminogen activator (tPA) leader sequence with full-length S (tPA.S)[12]; (ii) tPA leader sequence with full-length S and mutation of the furin cleavage site and two proline-stabilizing mutations (tPA.S.PP)[13–15]; (iii) wild-type leader sequence with native full-length S (S); (iv) wild-type leader sequence with S and deletion of the cytoplasmic tail (S.dCT)[16]; (v) tandem tPA and wild-type leader sequences with full-length S (tPA.WT.S)[12]; (vi) wild-type leader sequence with S with deletion of the transmembrane region and cytoplasmic tail reflecting the soluble ectodomain, with mutation of the furin cleavage site, proline-stabilizing mutations, and a foldon trimerization domain (S.dTM.PP)[15]; and (vii) wild-type leader sequence with full-length S and mutation of the furin cleavage site and proline-stabilizing mutations (S.PP) (Fig. 1a).

[1]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA. [2]Janssen Vaccines and Prevention BV, Leiden, The Netherlands. [3]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA. [4]Massachusetts Institute of Technology, Cambridge, MA, USA. [5]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. [6]Harvard Medical School, Boston, MA, USA. [7]Bioqual, Rockville, MD, USA. [8]Children's Hospital, Boston, MA, USA. [9]Massachusetts Consortium on Pathogen Readiness, Boston, MA, USA. [10]These authors contributed equally: Noe B. Mercado, Roland Zahn, Frank Wegmann, Carolin Loos, Abishek Chandrashekar, Jingyou Yu, Jinyan Liu, Lauren Peter, Katherine McMahan, Lisa H. Tostanoski, Xuan He, David R. Martinez. [11]These authors jointly supervised this work: Hanneke Schuitemaker, Dan H. Barouch. ✉e-mail: dbarouch@bidmc.harvard.edu

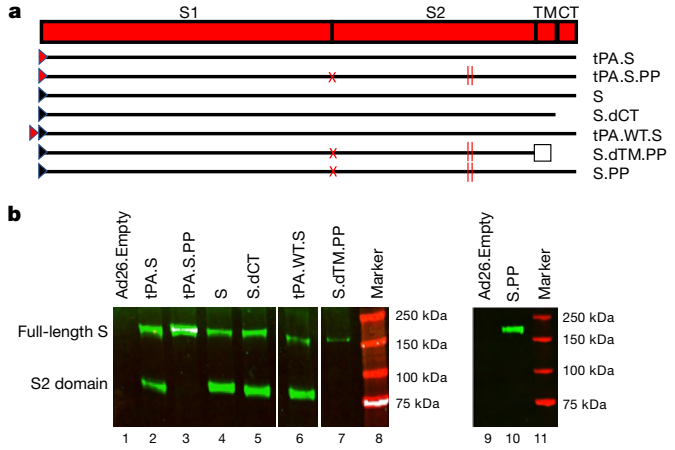

Fig. 1 | Construction of Ad26 vectors. a, Seven Ad26 vectors were produced that expressed SARS-CoV-2 S protein variants: (i) tPA leader sequence with full-length S (tPA.S)[12]; (ii) tPA leader sequence with full-length S with mutation of the furin cleavage site and two proline stabilizing mutations (tPA.S.PP)[13–15]; (iii) wild-type leader sequence with native full-length S (S); (iv) wild-type leader sequence with S with deletion of the cytoplasmic tail (S.dCT)[16]; (v) tandem tPA and wild-type leader sequences with full-length S as a strategy to enhance expression (tPA.WT.S)[12]; (vi) wild-type leader sequence with S with deletion of the transmembrane region and cytoplasmic tail, reflecting the soluble ectodomain, with mutation of the furin cleavage site, proline stabilizing mutations and a foldon trimerization domain (S.dTM.PP)[15]; and (vii) wild-type leader sequence with full-length S with mutation of the furin cleavage site and proline stabilizing mutations (S.PP). The red triangle depicts tPA leader sequence; black triangle depicts wild-type leader sequence; red X depicts furin cleavage site mutation; red vertical lines depict proline mutations; and open square depicts foldon trimerization domain. CT, cytoplasmic domain; S1 and S2, the first and second domain of the S protein; TM, transmembrane region. **b**, Western blot analyses for expression from Ad26 vaccine vectors encoding tPA.S (lane 2), tPA.S.PP (lane 3), S (lane 4), S.dCT (lane 5), tPA.WT.S (lane 6), S.dTM.PP (lane 7) or S.PP (lane 9) under non-reducing conditions in human MRC-5 cell lysates using a human monoclonal antibody (CR3046). This experiment was repeated three times. For gel source data, see Supplementary Fig. 1.

Western blot analyses confirmed expression of S in cell lysates from all vectors (Fig. 1b).

We immunized 52 adult rhesus macaques, aged 6–12 years old, with Ad26 vectors expressing tPA.S ($n = 4$), tPA.S.PP ($N = 4$), S ($n = 4$), S.dCT ($n = 4$), tPA.WT.S ($n = 4$), S.dTM.PP ($n = 6$) or S.PP ($n = 6$), and sham controls ($n = 20$). Macaques received a single immunization of 1×10^{11} viral particles of Ad26 vectors by the intramuscular route without adjuvant at week 0. We observed receptor-binding-domain (RBD)-specific binding antibodies by enzyme-linked immunosorbent assay (ELISA) in 31 out of 32 vaccinated macaques by week 2, and in all vaccinated macaques by week 4 (Fig. 2a). Neutralizing antibody responses were assessed using both a pseudovirus neutralization assay[9,10,16] (Fig. 2b) and a live virus neutralization assay[9,10,17,18] (Fig. 2c). Titres of neutralizing antibodies as measured by both assays were observed in most vaccinated macaques at week 2 and generally increased by week 4. The Ad26-S.PP vaccine elicited the highest titres of pseudovirus neutralizing antibodies (median 408, range 208–643) and live virus neutralizing antibodies (median 113, range 53–233) at week 4 ($P < 0.05$, two-sided Mann–Whitney tests). Titres of pseudovirus neutralizing antibodies correlated with both ELISA titres and live virus neutralizing antibody titres ($P < 0.0001$, $R = 0.8314$ and $P < 0.0001$, $R = 0.8427$, respectively, two-sided Spearman rank-correlation tests) (Extended Data Fig. 1). Median titres of neutralizing antibodies in the macaques vaccinated with Ad26-S.PP were fourfold higher than median titres of neutralizing antibodies in previously reported cohorts of 9 convalescent macaques[9] and 27 convalescent humans after recovery from SARS-CoV-2 infection[10] ($P < 0.0001$, two-sided Mann–Whitney test) (Extended Data Fig. 2a). The Ad26-S.PP vaccine also induced detectable S-specific IgG and IgA responses in bronchoalveolar lavage (BAL) samples (Extended Data Fig. 2b).

We further characterized S-specific and RBD-specific antibody responses in the vaccinated macaques by systems serology[19]. A variety of Fc effector functions, including antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent monocyte cellular phagocytosis (ADCP), antibody-dependent complement deposition (ADCD) and antibody-dependent natural killer cell activation (ADNKA), as well as several Ig subclasses and FcR binding were observed (Extended Data Fig. 3). The highest antibody-binding responses were observed with the Ad26-tPA.S.PP and Ad26-S.PP vaccines, and the highest effector function responses were seen with the Ad26-S.PP vaccine. A principal component analysis showed substantial overlap of the groups, although Ad26-S.PP was the most divergent group (Extended Data Fig. 3).

Cellular immune responses were induced in 30 out of 32 vaccinated macaques at week 4 by IFNγ enzyme-linked immune absorbent spot (ELISPOT) assays using pooled S peptides (Fig. 3a), and multiparameter intracellular cytokine staining assays were used to assess $IFN\gamma^+CD4^+$ and $CD8^+$ T cell responses (Fig. 3b). Responses were comparable across the vaccine groups. Analysis of a cohort of 10 similarly immunized macaques demonstrated that a single immunization of 1×10^{11} viral particles of Ad26-S.PP elicited consistent IFNγ ELISPOT responses but minimal or no IL-4 ELISPOT responses (Extended Data Fig. 4), which suggests induction of type 1 T helper (T_H1)-biased responses.

Protective efficacy of Ad26 vaccine candidates

At week 6, all macaques were challenged with 1.0×10^5 50% tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2 by the intranasal and intratracheal routes[9,10]. Consistent with previous observations, clinical disease was minimal in all macaques after challenge[9,10]. Viral loads in BAL and nasal swabs were assessed by reverse transcription PCR (RT–PCR) specific for subgenomic mRNA (sgRNA), which is thought to measure replicating virus[9,20]. All 20 sham controls were infected and showed a median peak of 4.89 (range 3.85–6.51) $\log_{10}$[sgRNA (copies per ml)] in BAL samples (Fig. 4a). By contrast, macaques that were treated with Ad26-S.PP had no detectable virus in BAL samples. Partial protection was observed with the other vaccines, with occasional macaques showing low levels of sgRNA in BAL (Fig. 4b). Similarly, sham controls showed a median peak of 5.59 (range 3.78–8.01) $\log_{10}$[sgRNA (copies per swab)] in nasal swabs (Fig. 4c). Only one of the macaques that received the Ad26-S.PP vaccine showed a low amount of virus in nasal swabs. The macaques that were treated with the other vaccines generally had reduced viral loads in nasal swabs compared with control macaques, although protection was optimal with Ad26-S.PP (Fig. 4d). All vaccinated macaques showed no detectable infectious virus in nasal swabs by plaque-forming unit (PFU) assays (Extended Data Fig. 5).

A comparison of peak viral loads in the vaccinated macaques suggested that protection in BAL samples was generally more robust than in nasal swabs (Fig. 5). The Ad26-S.PP vaccine provided complete protection in both the lower and upper respiratory tract with the exception of one macaque that showed a low amount of virus in nasal swabs, and resulted in greater than 3.2 and 3.9 $\log_{10}$-transformed reductions of median peak sgRNA in BAL and nasal swabs, respectively, as compared with sham controls ($P < 0.0001$ and $P < 0.0001$, respectively, two-sided Mann–Whitney tests) (Fig. 5). Among the 32 vaccinated macaques, 17 were completely protected and had no detectable sgRNA in BAL or nasal swabs after challenge, and 5 additional macaques had no sgRNA in BAL but showed some virus in nasal swabs.

Immune correlates of protection

The size of this study and the variability in outcomes with the different vaccine constructs facilitated an immune correlates analysis. The $\log_{10}$(ELISA titre), $\log_{10}$(pseudovirus neutralizing antibody titre) and

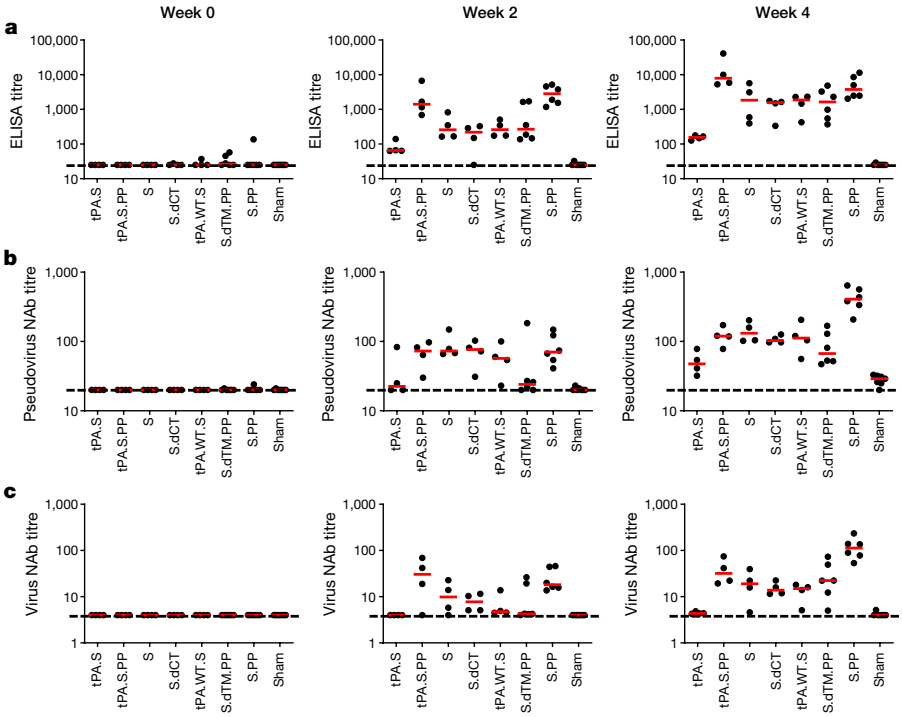

Fig. 2 | Humoral immune responses in vaccinated rhesus macaques. **a**–**c**, Humoral immune responses were assessed at weeks 0, 2 and 4 by a RBD-specific binding antibody ELISA (**a**), pseudovirus neutralization assays (**b**), and live virus neutralization assays (**c**). Red bars reflect median responses. Dotted lines reflect assay limit of quantification. NAb, neutralizing antibody.

$\log_{10}$(live virus neutralizing antibody titre) at weeks 2 and 4 inversely correlated with peak $\log_{10}$(sgRNA) in both BAL (Fig. 6) and nasal swabs (Extended Data Fig. 6). In general, week-4 titres correlated better than week-2 titres, and neutralizing antibody titres correlated better than ELISA titres. The $\log_{10}$(pseudovirus neutralizing antibody titre) and $\log_{10}$(live virus neutralizing antibody titre) at week 4 inversely correlated with peak $\log_{10}$(sgRNA) in BAL ($P < 0.0001$, $R = -0.6880$ and $P < 0.0001$, $R = -0.6562$, respectively, two-sided Spearman rank-correlation test) (Fig. 6b, c) and in nasal swabs ($P < 0.0001$, $R = -0.5839$, and $P < 0.0001$, $R = -0.5714$, respectively, two-sided Spearman rank-correlation test) (Extended Data Fig. 6b, c). Together with previously published data[10], these findings suggest that serum antibody titres may prove a useful immune correlate of protection for SARS-CoV-2 vaccines. By contrast, vaccine-elicited ELISPOT responses, and $CD4^+$ and $CD8^+$ intracellular cytokine staining responses, did not correlate with protection (data not shown).

To gain further insight into antibody correlates of protection, we defined antibody parameters that distinguished completely protected macaques (defined as macaques with no detectable sgRNA in BAL or nasal swabs after challenge) and partially protected or non-protected macaques. The neutralizing antibody titre was the parameter most enriched in completely protected macaques compared with partially protected or non-protected macaques ($P = 0.0009$, two-sided Mann–Whitney test), followed by ADNKA ($P = 0.0044$) and ADCP ($P = 0.0092$) responses (Fig. 6d). Moreover, a logistic regression analysis showed that using two features, such as neutralizing antibody titre and FcγR2A-3, IgM or ADCD responses, improved correlation with protection (Fig. 6d). These data suggest that neutralizing antibodies are primarily responsible for protection against SARS-CoV-2 but that other binding and functional antibodies may also be involved.

Immune responses in vaccinated macaques after challenge

Sham controls and most of the vaccinated macaques (excluding Ad26-S.PP-vaccinated macaques) developed substantially higher pseudovirus neutralizing antibody responses (Extended Data Figs. 7, 8) as well as $CD8^+$ and $CD4^+$ T cell responses (Extended Data Fig. 9) by day 14 after SARS-CoV-2 challenge. $CD8^+$ and $CD4^+$ T cell responses were directed against several SARS-CoV-2 proteins, including spike (S1 and S2), nucleocapsid (NCAP), and non-structural (NS6, NS7a and NS8) proteins, in the sham controls. By contrast, macaques that were treated with the Ad26-S.PP vaccine did not show anamnestic neutralizing antibody responses (Extended Data Fig. 7) and only had low T cell responses against spike proteins (S1 and S2) (Extended Data Fig. 9), which was the vaccine antigen, after challenge. These findings are consistent with the largely undetectable viral loads in the Ad26-S.PP-vaccinated macaques (Figs. 4, 5) and suggest exceedingly low levels of viral replication in these macaques, if any at all, after challenge. Immunophenotyping of BAL

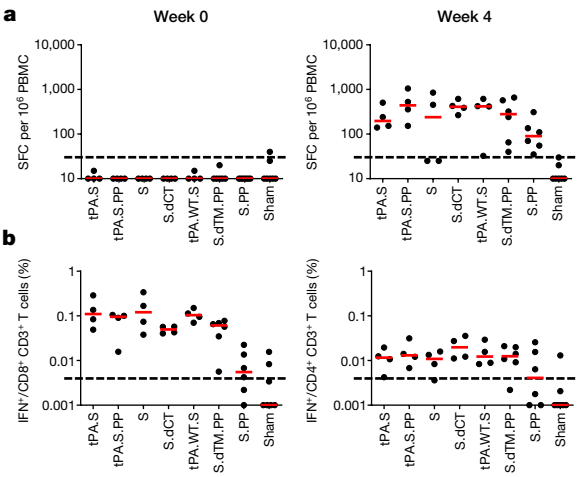

Fig. 3 | Cellular immune responses in vaccinated rhesus macaques. **a**, **b**, Cellular immune responses were assessed at week 4 after immunization by IFNγ ELISPOT assays (**a**) and $IFN\gamma^+CD4^+$ and $IFN\gamma^+CD8^+$ T cell intracellular cytokine staining assays (**b**) in response to pooled S peptides. Red bars reflect median responses. Dotted lines reflect assay limit of quantification.

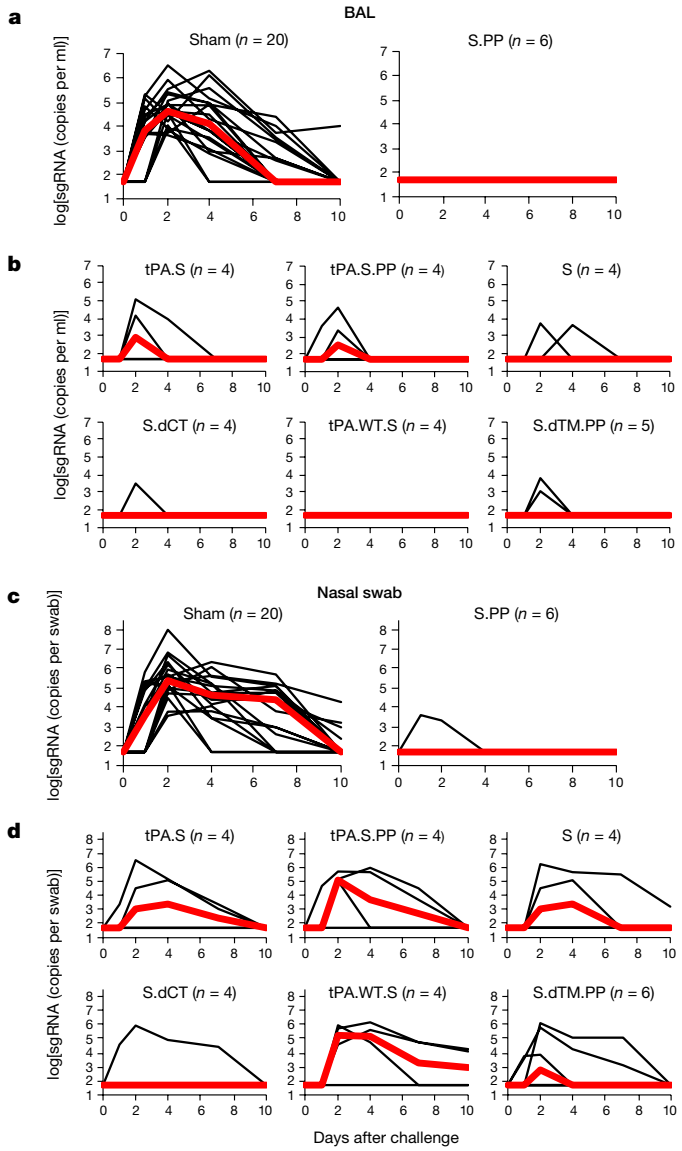

Fig. 4 | Viral loads in rhesus macaques after SARS-CoV-2 challenge. Rhesus macaques were challenged by the intranasal and intratracheal routes with 1.0×10^5 $TCID_{50}$ of SARS-CoV-2. **a**, **b**, log_{10}[sgRNA (copies per ml)] (limit of quantification 50 copies per ml) were assessed in BAL samples in sham controls and vaccinated macaques after challenge. **c**, **d**, log_{10}[sgRNA (copies per swab)] (limit of quantification 50 copies per swab) were assessed in nasal swabs (NS) in sham controls and vaccinated macaques after challenge. Days after challenge are shown on the *x* axis. One macaque in the S.dTM.PP group did not have peak BAL samples obtained after challenge. Red lines reflect median values.

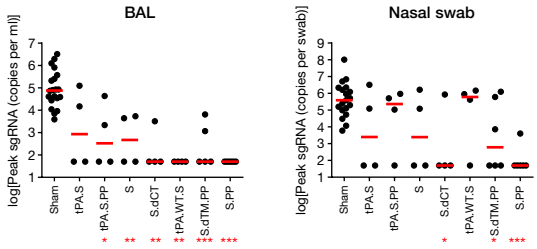

Fig. 5 | Summary of peak viral loads after SARS-CoV-2 challenge. Peak viral loads in BAL and nasal swabs after challenge. Peak viral loads occurred variably on days 1–4 after challenge. Red lines reflect median viral loads. $^*P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.0001$, two-sided Mann–Whitney test. $n = 4$, 5 or 6 biologically independent macaques in vaccine groups and $n = 20$ biologically independent macaques in sham group.

cells from these macaques suggested similar cellular subpopulations in vaccinated and control macaques after immunization and challenge (Extended Data Fig. 10).

Discussion

The development of a safe and effective SARS-CoV-2 vaccine is a crucial global priority. Our data demonstrate that a single immunization with an Ad26 vector encoding a prefusion stabilized S immunogen (S.PP) induced robust neutralizing antibody responses and provided complete protection against SARS-CoV-2 challenge in five out of six rhesus macaques and near-complete protection in one out of six macaques. The S.PP immunogen contains the wild-type leader sequence, the full-length membrane-bound S protein, mutation of the furin cleavage site and two proline-stabilizing mutations[15].

Our data extend recent preclinical studies of inactivated virus vaccines and DNA vaccines for SARS-CoV-2 in non-human primates[10,21]. Whereas inactivated virus vaccines and nucleic acid vaccines typically require two or more immunizations, some adenovirus vectors can induce robust and durable neutralizing antibody responses after a single immunization[22–24]. A single-shot SARS-CoV-2 vaccine would have important logistical and practical advantages compared with a two-dose vaccine for mass vaccination campaigns and control of the pandemic. However, we would expect that a two-dose vaccine with Ad26-S.PP would be more immunogenic. Our previous data demonstrate that a homologous boost with Ad26-HIV vectors augmented antibody titres by more than tenfold in both non-human primates and humans[25–27], which suggests that both single-dose and two-dose regimens of the Ad26-S.PP vaccine should be evaluated in clinical trials. Baseline Ad26 neutralizing antibody titres in human populations[11,28] have not suppressed the immunogenicity of an Ad26-HIV vaccine in several geographical regions[25], suggesting the generalizability of this vector platform; however, this will be evaluated in clinical trials that are now underway.

Ad26-S.PP induced robust neutralizing antibody responses after a single immunization and provided complete protection against SARS-CoV-2 challenge in five out of six macaques, whereas one macaque had low levels of virus in nasal swabs. It is important to note that in the Ad26-S.PP-vaccinated macaques, neutralizing antibody titres and T cell responses did not expand after challenge, and T cell responses also did not broaden to non-vaccine antigens such as nucleocapsid and non-structural proteins. By contrast, sham controls and macaques that were treated with the other vaccines generated higher titres of neutralizing antibodies and T cell responses to several SARS-CoV-2 proteins after challenge, consistent with previous observations with DNA vaccines[10]. These data suggest minimal to no virus replication in the Ad26-S.PP-vaccinated macaques after SARS-CoV-2 challenge.

Vaccine-elicited titres of neutralizing antibodies before challenge correlated with protection in both BAL and nasal swabs after challenge, consistent with previous findings[10]. These data suggest that serum titres of neutralizing antibodies may be a potential biomarker for vaccine protection, although this will need to be confirmed in additional SARS-CoV-2 vaccine efficacy studies in both non-human primates and humans. Moreover, further functional antibody responses may also contribute to protection, such as ADNKA, ADCP and ADCD responses. The role of T cell responses in vaccine protection remains to be determined.

A limitation of our study is that we did not evaluate the durability of neutralizing antibody responses elicited by these vaccines, and future studies are planned to investigate this question. Additional

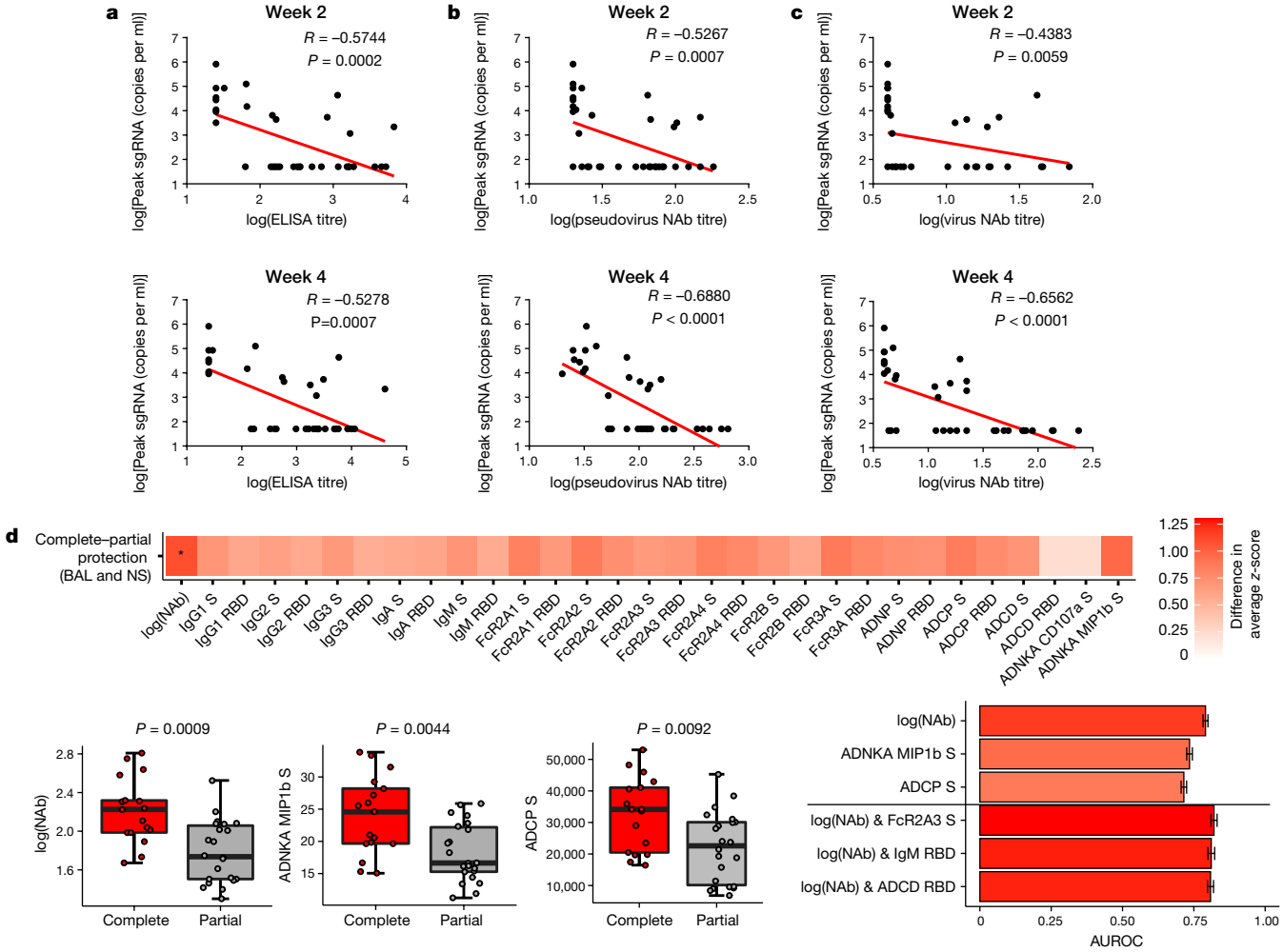

Fig. 6 | Antibody correlates of protection. a–d, Correlations of binding ELISA titres (**a**), pseudovirus neutralizing antibody titres (**b**) and live virus neutralizing antibody titres (**c**) at weeks 2 and 4 with log[peak sgRNA (copies per ml)] in BAL after challenge. Red lines reflect the best linear fit relationship between these variables. *P* and *R* values reflect two-sided Spearman rank-correlation tests. $n = 52$ biologically independent macaques. **d**, The heat map shows the differences in the means of *z*-scored features between completely protected ($n = 17$) and partially protected and non-protected ($n = 22$) macaques. The two groups were compared by two-sided Mann–Whitney tests, and asterisks indicate the Benjamini–Hochberg corrected *q*-values (*$q < 0.05$), with $q = 0.02707$ for log(neutralizing antibodies). The dot plots show differences in the features that best discriminated completely protected and partially protected macaques, including neutralizing antibody titres, S-specific ADNKA and ADCP responses. *P* values were determined by two-sided Mann–Whitney tests. For the box plots, the upper bound of the box indicates the 75th percentile, and the lower bound the 25th percentile. The horizontal line shows the median, and the whiskers indicate minimum and maximum values. The bar plot shows the cross-validated area under the receiver operator characteristics curves using the features indicated on the *x* axis in a logistic regression model. The top three one-feature and two-feature models are shown. AUROC, areas under the receiver operator characteristics. Data are mean ± s.d. for 100 repetitions of tenfold cross-validation.

studies could also evaluate mucosal delivery of this vaccine. Our studies were also not specifically designed to assess safety or the possibility of vaccine-associated enhanced respiratory disease or antibody-dependent enhancement of infection[29]. However, it is worth noting that the Ad26-S.PP vaccine elicited T_H1-biased rather than T_H2-biased T cell responses, and macaques with sub-protective neutralizing antibody titres did not demonstrate enhanced viral replication or clinical disease. Moreover, immunophenotyping of BAL cell subpopulations did not reveal increased eosinophils in vaccinated macaques compared with control macaques after immunization or challenge.

In summary, our data demonstrate that a single immunization of Ad26 vector-based vaccines for SARS-CoV-2 elicited robust neutralizing antibody titres and provided complete or near-complete protection against SARS-CoV-2 challenge in rhesus macaques. It is likely that protection in both the upper and lower respiratory tracts will be required to prevent transmission and disease in humans. The identification of a neutralizing antibody correlate of protection should prove useful in the clinical development of SARS-CoV-2 vaccines. The optimal Ad26-S.PP vaccine from this study, termed Ad26.COV2.S, is currently being evaluated in clinical trials.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2607-z.

1. Wu, F. et al. A new coronavirus associated with human respiratory disease in China. *Nature* **579**, 265–269 (2020).
2. Zhou, P. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270–273 (2020).

3. Holshue, M. L. et al. First case of 2019 novel coronavirus in the United States. *N. Engl. J. Med*. **382**, 929–936 (2020).
4. Li, Q. et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N. Engl. J. Med*. **382**, 1199–1207 (2020).
5. Zhu, N. et al. A Novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med*. **382**, 727–733 (2020).
6. Chen, N. et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* **395**, 507–513 (2020).
7. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497–506 (2020).
8. Chan, J. F. et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* **395**, 514–523 (2020).
9. Chandrashekar, A. et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. *Science* **369**, 812–817 (2020).
10. Yu, J. et al. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science* **369**, 806–811 (2020).
11. Abbink, P. et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J. Virol*. **81**, 4654–4663 (2007).
12. Alharbi, N. K. et al. ChAdOx1 and MVA based vaccine candidates against MERS-CoV elicit neutralising antibodies and cellular immune responses in mice. *Vaccine* **35**, 3780–3788 (2017).
13. Kirchdoerfer, R. N. et al. Pre-fusion structure of a human coronavirus spike protein. *Nature* **531**, 118–121 (2016).
14. Pallesen, J. et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proc. Natl Acad. Sci. USA* **114**, E7348–E7357 (2017).
15. Wrapp, D. et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260–1263 (2020).
16. Yang, Z. Y. et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature* **428**, 561–564 (2004).
17. Scobey, T. et al. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. *Proc. Natl Acad. Sci. USA* **110**, 16157–16162 (2013).
18. Yount, B. et al. Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. *Proc. Natl Acad. Sci. USA* **100**, 12995–13000 (2003).
19. Chung, A. W. et al. Dissecting polyclonal vaccine-induced humoral immunity against HIV using systems serology. *Cell* **163**, 988–998 (2015).
20. Wölfel, R. et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* **581**, 465–469 (2020).
21. Gao, Q. et al. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* **369**, 77–81 (2020).
22. Abbink, P. et al. Durability and correlates of vaccine protection against Zika virus in rhesus monkeys. *Sci. Transl. Med*. **9**, eaao4163 (2017).
23. Abbink, P. et al. Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. *Science* **353**, 1129–1132 (2016).
24. Cox, F. et al. Adenoviral vector type 26 encoding Zika virus (ZIKV) M-Env antigen induces humoral and cellular immune responses and protects mice and nonhuman primates against ZIKV challenge. *PLoS ONE* **13**, e0202820 (2018).
25. Barouch, D. H. et al. Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). *Lancet* **392**, 232–243 (2018).
26. Barouch, D. H. et al. Protective efficacy of adenovirus/protein vaccines against SIV challenges in rhesus monkeys. *Science* **349**, 320–324 (2015).
27. Baden, L. R. et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). *J. Infect. Dis*. **207**, 240–247 (2013).
28. Barouch, D. H. et al. International seroepidemiology of adenovirus serotypes 5, 26, 35, and 48 in pediatric and adult populations. *Vaccine* **29**, 5203–5209 (2011).
29. Graham, B. S. Rapid COVID-19 vaccine development. *Science* **368**, 945–946 (2020).

Methods

No statistical methods were used to predetermine sample sizes. Cell lines were purchased from ATCC, and tested for mycoplasma.

Animals and study design

In total, 52 outbred Indian-origin adult male and female rhesus macaques (*M. mulatta*), 6–12 years old, were randomly allocated to groups. All macaques were housed at Bioqual. Macaques were treated with Ad26 vectors expressing tPA.S ($n = 4$), tPA.S.PP ($n = 4$), S ($n = 4$), S.dCT ($n = 4$), tPA.WT.S ($n = 4$), S.dTM.PP ($n = 6$) or S.PP ($n = 6$), and sham controls ($n = 20$). Macaques received a single immunization of 10^{11} viral particles of Ad26 vectors by the intramuscular route without adjuvant at week 0. At week 6, all macaques were challenged with 1.0×10^5 $TCID_{50}$ (1.2×10^8 RNA copies, 1.1×10^4 PFU) SARS-CoV-2, which was derived from USA-WA1/2020 (NR-52281; BEI Resources)[9]. Viral particle titres were assessed by RT–PCR. Virus was administered as 1 ml by the intranasal route (0.5 ml in each nare) and 1 ml by the intratracheal route. All immunological and virological assays were performed blinded. All animal studies were conducted in compliance with all relevant local, state and federal regulations and were approved by the Bioqual Institutional Animal Care and Use Committee (IACUC).

Ad26 vectors

Ad26 vectors were constructed with seven variants of the SARS-CoV-2 spike (S) protein sequence (Wuhan/WIV04/2019; GenBank MN996528.1). Sequences were codon-optimized and synthesized. Replication-incompetent, E1/E3-deleted Ad26-vectors[11] were produced in PER.C6.TetR cells using a plasmid containing the full Ad26 vector genome and a transgene expression cassette. Vectors were sequenced and tested for expression before use.

Western blot

For western blot analysis, 24-well plates were seeded with MRC-5 cells (1.25×10^5 cells per well), and after overnight growth they were transduced with Ad26 vectors encoding SARS-CoV-2 Spike transgenes. Cell lysates were collected 48 h after transduction and, after heating for 5 min at 85 °C, samples were loaded under non-reduced conditions on a precast 4–12% Bis-Tris SDS–PAGE gel (Invitrogen). Proteins were transferred to a nitrocellulose membrane using an iBlot2 dry blotting system (Invitrogen), and membrane blocking was performed overnight at 4 °C in Tris-buffered saline (TBS) containing 0.2% Tween 20 (v/v) (TBST) and 5% (w.v) Blotting-Grade Blocker (Bio-Rad). After overnight blocking, the membrane was incubated for 1 h with 2.8 μg ml^{-1} CR3046 in TBST-5% blocker. CR3046 is a human monoclonal antibody directed against SARS-CoV spike and binds to the spike S2 domain, and also cross-reacts with SARS-CoV-2 spike S2 (unpublished data). After incubation, the membrane was washed three times with TBST for 5 min and subsequently incubated for 1 h with 1:10,000 IRDye 800CW-conjugated goat-anti-human secondary antibody (Li-COR) in TBST-5% blocker. Finally, the PVDF membrane was washed three times with TBST for 5 min, and after drying developed using an ODYSSEY CLx Infrared Imaging System (Li-COR).

Subgenomic mRNA assay

SARS-CoV-2 *E* gene sgRNA was assessed by RT–PCR using primers and probes as previously described[9,10,20]. In brief, to generate a standard curve, the SARS-CoV-2 *E* gene sgRNA was cloned into a pcDNA3.1 expression plasmid; this insert was transcribed using an AmpliCap-Max T7 High Yield Message Maker Kit (Cellscript) to obtain RNA for standards. Before RT–PCR, samples collected from challenged macaques or standards were reverse-transcribed using Superscript III VILO (Invitrogen) according to the manufacturer's instructions. A Taqman custom gene expression assay (ThermoFisher Scientific) was designed using the sequences targeting the *E* gene sgRNA[20]. Reactions were carried out on a QuantStudio 6 and 7 Flex Real-Time PCR System (Applied Biosystems) according to the manufacturer's specifications. Standard curves were used to calculate sgRNA in copies per ml or per swab; the quantitative assay sensitivity was 50 copies per ml or per swab.

PFU assay

For plaque assays, confluent monolayers of Vero E6 cells (ATCC) were prepared in 6-well plates. Indicated samples collected from challenged macaques were serially diluted, added to wells, and incubated at 37 °C for 1 h. After incubation, 1.5 ml of 0.5% methylcellulose medium was added to each well and the plates were incubated at 37 °C with 5% CO_2 for 2 days. Plates were fixed by adding 400 μl ice-cold methanol per well and incubating at −20 °C for 30 min. After fixation, the methanol was discarded, and cell monolayers were stained with 600 μl per well of 0.23% crystal violet for 30 min. After staining, the crystal violet was discarded, and the plates were washed once with 600 μl water to visualize and count plaques.

ELISA

RBD-specific binding antibodies were assessed by ELISA as previously described[9,10]. In brief, 96-well plates were coated with 1 μg ml^{-1} SARS-CoV-2 RBD protein (A. Schmidt, MassCPR) in 1× DPBS and incubated at 4 °C overnight. After incubation, plates were washed once with wash buffer (0.05% Tween 20 in 1× DPBS) and blocked with 350 μl casein block per well for 2–3 h at room temperature. After incubation, block solution was discarded and plates were blotted dry. Serial dilutions of heat-inactivated serum diluted in casein block were added to wells and plates were incubated for 1 h at room temperature, before three further washes and a 1-h incubation with a 1:1,000 dilution of anti-macaque IgG HRP (NIH NHP Reagent Program) at room temperature in the dark. Plates were then washed three times, and 100 μl of SeraCare KPL TMB SureBlue Start solution was added to each well; plate development was halted by the addition of 100 μl SeraCare KPL TMB Stop solution per well. The absorbance at 450 nm was recorded using a VersaMax or Omega microplate reader. ELISA endpoint titres were defined as the highest reciprocal serum dilution that yielded an absorbance >0.2. The $\log_{10}$(endpoint titres) are reported.

Pseudovirus neutralization assay

The SARS-CoV-2 pseudoviruses expressing a luciferase reporter gene were generated in an similar approach to that previously described[9,10,16]. In brief, the packaging construct psPAX2 (AIDS Resource and Reagent Program), luciferase reporter plasmid pLenti-CMV Puro-Luc (Addgene), and spike protein expressing pcDNA3.1-SARS-CoV-2 SΔCT were co-transfected into HEK293T cells with calcium phosphate. The supernatants containing the pseudotype viruses were collected 48 h after transfection; pseudotype viruses were purified by filtration with 0.45-μm filter. To determine the neutralization activity of the antisera from vaccinated macaques, HEK293T-hACE2 cells were seeded in 96-well tissue culture plates at a density of 1.75×10^4 cells per well overnight. Twofold serial dilutions of heat-inactivated serum samples were prepared and mixed with 50 μl of pseudovirus. The mixture was incubated at 37 °C for 1 h before adding to HEK293T-hACE2 cells. After 48 h, cells were lysed in Steady-Glo Luciferase Assay (Promega) according to the manufacturer's instructions. SARS-CoV-2 neutralization titres were defined as the sample dilution at which a 50% reduction in relative light units was observed relative to the average of the virus control wells.

Live virus neutralization assay

A full-length SARS-CoV-2 virus based on the Seattle Washington isolate was designed to express nanoluciferase (nLuc) and GFP and was recovered via reverse genetics and previously described[17,18]. The SARS-CoV-2 nLuc-GFP virus titre was measured in Vero E6 USAMRIID cells, as defined by PFU per ml, in a 6-well plate format in quadruplicate biological replicates for accuracy. In addition, the virus was titred in Vero E6 USAMRID

cells to ensure the relative light unit signal was at least ten times the cell only control background. For the 96-well neutralization assay, Vero E6 USAMRID cells were plated at 20,000 cells per well the day before in clear-bottom black-walled plates. Cells were inspected to ensure confluency on the day of assay. In separate 96-well dilution plates, neutralizing antibody serum samples were diluted to a starting dilution of 1:4 and were serially diluted fourfold up to eight dilution spots. Serially diluted serum samples were added in equal volume to 90 PFU of virus in duplicate test wells. Cell and virus-only control wells were also included in each 96-well dilution plate. The antibody–virus and virus-only mixtures were then incubated at 37 °C with 5% CO_2 for exactly 1 h. After incubation, growth medium was removed from the clear-bottom black-walled 96-well plates and virus–antibody dilution complexes and virus-only and cell controls were added to the cells in duplicate and tips were replaced between each duplicate sample. After infection, 96-well neutralization assay plates were incubated at 37 °C with 5% CO_2 for 48 h. After the 48-h incubation, cells were lysed, and luciferase activity was measured via Nano-Glo Luciferase Assay System (Promega) according to the manufacturer specifications. Luminescence was measured by a Spectramax M3 plate reader (Molecular Devices). SARS-CoV-2 neutralization titres were defined as the sample dilution at which a 50% reduction in relative light units was observed relative to the average of the virus control wells.

Systems serology

Luminex. A customized multiplexed approach to quantify relative antigen-specific antibody titres was used, as previously described[30]. Therefore, microspheres (Luminex) with a unique fluorescence were coupled with SARS-CoV-2 antigens including S protein and RBD via covalent *N*-hydroxysuccinimide (NHS)–ester linkages via EDC (Thermo Scientific) and Sulfo-NHS (Thermo Scientific). Per well in a 384-well plate (Greiner), 1.2×10^3 beads per region or antigen were added and incubated with diluted serum sample (1:100 for all isotypes or subclasses except for IgG1, which was diluted 1:250 as well as Fc-receptor binding) for 16 h shaking at 900 rpm at 4 °C. After formation of immune complexes, microspheres were washed three times in 0.1% BSA and 0.05% Tween 20 (Luminex assay buffer) with an automated plate washer (Tecan). Anti-rhesus IgG1, IgG2, IgG3, IgA (NIH NHP Reagent Program) and IgM (Life Diagnostic) detection antibodies were diluted in Luminex assay buffer to 0.65 μg ml^{-1} and incubated with beads for 1 h at room temperature while shaking at 900 rpm. After washing of stained immune complexes, a tertiary goat anti-mouse IgG-PE antibody (Southern Biotech) was added to each well at 0.5 μg ml^{-1} and incubated for 1 h at room temperature on a shaker. Similarly, for the Fc-receptor binding profiles, recombinant rhesus FcγR2A-1, FcγR2A-2, FcγR2A-3, FcγR2A-4, FcγR3A and human FcγR2B (Duke Protein Production facility) were biotinylated (Thermo Scientific) and conjugated to Streptavidin-PE for 10 min (Southern Biotech). The coated beads were then washed and read on a flow cytometer, iQue (Intellicyt) with a robot arm attached (PAA). Events were gated on each bead region, and the median fluorescence of phycoerythrin (PE) for bead-positive events was reported. Samples were run in duplicate per each secondary detection agent.

ADNP, ADCP and ADCD assays. ADNP, ADCP and ADCD assays were performed as previously described[31–33]. In brief, SARS-CoV-2 S and RBD were biotinylated (Thermo Fisher) and coupled to 1 μm yellow (ADCP and ADNP) and red (ADCD) fluorescent beads for 2 h at 37 °C. Excess antigen was removed by washing twice with 0.1% BSA in PBS. Next, 1.82×10^8 antigen-coated beads were added to each well of a 96-well plate and incubated with diluted samples (ADCP and ADNP 1:100, ADCD 1:10) at 37 °C for 2 h to facilitate immune complex formation. After the incubation, complexed beads were washed and for ADCP assays, 2.5×10^4 THP-1 cells (American Type Culture Collection) were added per well and incubated for 16 h at 37 °C. For ADNP assays, peripheral blood mononuclear cells (PBMCs) were isolated from healthy blood donors by lysis of red blood cells by addition of Ammonium-Chloride-Potassium (ACK) lysis (Thermo Fisher) and 5×10^4 cells were added per well and incubated for 1 h at 37 °C. Subsequently, primary blood cells were stained with an anti-Cd66b Pac blue detection antibody (BioLegend). For ADCD assays, lyophilized guinea pig complement was reconstituted according to manufacturer's instructions (Cedarlane) with water and 4 μl per well were added in gelatin veronal buffer containing Mg^{2+} and Ca^{2+} (GVB^{++}, Boston BioProducts) to the immune complexes for 20 min at 37 °C. After washing twice with 15 mM EDTA in PBS, immune complexes were stained with a fluorescein-conjugated goat IgG fraction to guinea pig complement C3 (MpBio). After incubation with THP-s and staining of cells for ADNP and ADCD cell samples are fixed with 4% paraformaldehyde and sample acquisition was performed via flow cytometry (Intellicyt, iQue Screener plus) using a robot arm (PAA). All events were gated on single cells and bead-positive events, for ADCP and ADNP assays, a phagocytosis score was calculated as the percentage of bead positive cells × GMFI/1,000; in which GMFI denotes geometric mean fluorescence intensity. For ADCD assays, the median of C3-positive events is reported. All samples were run in duplicate on separate days.

ADNKA assay. For analysis of natural killer cell-related responses, an ELISA-based assay was used. Therefore, 96-well ELISA plates (Thermo Fisher) were coated with SARS-CoV-2 S at 37 °C for 2 h. Plates were then washed and blocked with 5% BSA in PBS overnight at 4 °C. Natural killer cells were isolated from buffy coats from healthy donors (MGH blood donor centre) using the RosetteSep isolation kit (Stem Cell Technologies) and natural killer cells were rested overnight supplemented with IL-15 (Stemcell). Serum samples were diluted 1:50 and incubated at 37 °C for 2 h on the ELISA plates. A staining cocktail of anti-CD107a-PE-Cy5 stain (BD), brefeldin A (Sigma), and GolgiStop (BD) was added to the natural killer cells and 5×10^4 natural killer cells per well were added and incubated for 5 h at 37 °C. Natural killer cells were fixed and permeabilized using Perm A and B (Thermo Fisher) and surface markers were stained for with anti-CD16 APC-Cy7 (BD), anti-CD56 PE-Cy7 (BD) and anti-CD3 AlexaFluor 700 antibodies (BD). Intracellular staining included anti-IFNγ APC (BD) and anti-MIP-1β PE (BD). Acquisition occurred by flow cytometry iQue (Intellicyt), equipped with a robot arm (PAA). Natural killer cells were defined as $CD3^-$, $CD16^+$ and $CD56^+$. The ADNKA assay was performed in duplicate across two blood donors.

Analysis. All isotypes or subclasses, Fc-receptor binding and ADCD data were $\log_{10}$-transformed. For the radar plots, each antibody feature was normalized such that its minimal value is 0 and the maximal value is 1 across groups before using the median within a group. A principal component analysis (PCA) was constructed using the R package 'ropls' to compare multivariate profiles. Completely protected macaques were defined as having no detectable sgRNA copies per ml in BAL and nasal swabs. Completely protected versus partially protected and non-protected macaques were compared using two-sided Mann–Whitney tests. For the visualization in the heat map, the differences in the means of completely and partially protected group of *z*-scored features were shown. To indicate significances in the heat map, a Benjamini–Hochberg correction was used to correct for multiple comparisons. To assess the ability of features and their combinations to predict protection, logistic regression models were trained for 100 repetitions in a tenfold cross-validation framework and areas under the receiver operator characteristics curves were calculated. All potential combinations of two features were tested. For this, the R packages glm and pROC were used.

IFNγ ELISPOT assay

ELISPOT plates were coated with mouse anti-human IFNγ monoclonal antibody from BD Pharmingen at a concentration of 5 μg per well overnight at 4 °C. Plates were washed with DPBS containing 0.25% Tween 20, and blocked with R10 medium (RPMI with 11% FBS and 1.1%

penicillin-streptomycin) for 1 h at 37 °C. The spike 1 and spike 2 peptide pools contain 15 amino acid peptides overlapping by 11 amino acids that span the protein sequence and reflect the N- and C-terminal halves of the protein, respectively. Spike 1 and 2 peptide pools were prepared at a concentration of 2 µg per well, and 200,000 cells per well were added. The peptides and cells were incubated for 18–24 h at 37 °C. All steps following this incubation were performed at room temperature. The plates were washed with coulter buffer and incubated for 2 h with rabbit polyclonal anti-human IFNγ biotin from U-Cytech (1 µg ml^{-1}). The plates are washed a second time and incubated for 2 h with Streptavidin-alkaline phosphatase antibody from Southern Biotechnology (1 µg ml^{-1}). The final wash was followed by the addition of Nitor-blue Tetrazolium Chloride/5-bromo-4-chloro 3 'indolyl phosphate p-toludine salt (NBT/BCIP chromagen) substrate solution for 7 min. The chromagen was discarded and the plates were washed with water and dried in a dim place for 24 h. Plates were scanned and counted on a Cellular Technologies Limited Immunospot Analyzer.

IL-4 ELISPOT assay

Pre-coated monoclonal antibody IL-4 ELISPOT plates (Mabtech) were washed and blocked. The assay was then performed as described above except the development time with NBT/BCIP chromagen substrate solution was 12 min.

Intracellular cytokine staining assay

Approximately 10^6 PBMCs per well were re-suspended in 100 µl of R10 medium supplemented with CD49d monoclonal antibody (1 µg ml^{-1}). Each sample was assessed with mock (100 µl of R10 plus 0.5% DMSO; background control), peptide pools (2 µg ml^{-1}), or 10 pg ml^{-1} phorbol myristate acetate (PMA) and 1 µg ml^{-1} ionomycin (Sigma-Aldrich) (100 µl; positive control) and incubated at 37 °C for 1 h. After incubation, 0.25 µl of GolgiStop and 0.25 µl of GolgiPlug in 50 µl of R10 was added to each well and incubated at 37 °C for 8 h and then held at 4 °C overnight. The next day, the cells were washed twice with DPBS, stained with Near IR live/dead dye for 10 min and then stained with predetermined titres of monoclonal antibodies against CD279 (clone EH12.1, BB700), CD38 (clone OKT10, PE), CD28 (clone 28.2, PE CY5), CD4 (clone L200, BV510), CD45 (clone D058-1283, BUV615), CD95 (clone DX2, BUV737), CD8 (clone SK1, BUV805), for 30 min. Cells were then washed twice with 2% FBS/DPBS buffer and incubated for 15 min with 200 µkl of BD CytoFix/CytoPerm Fixation/Permeabilization solution. Cells were washed twice with 1× Perm Wash buffer (BD Perm/Wash Buffer 10× in the CytoFix/CytoPerm Fixation/ Permeabilization kit diluted with MilliQ water and passed through 0.22-µm filter) and stained with intracellularly with monoclonal antibodies against Ki67 (clone B56, FITC), CD69 (clone TP1.55.3, ECD), IL10 (clone JES3-9D7, PE CY7), IL-13 (clone JES10-5A2, BV421), TNF (clone Mab11, BV650), IL-4 (clone MP4-25D2, BV711), IFNγ (clone B27; BUV395), IL-2 (clone MQ1-17H12, APC), CD3 (clone SP34.2, Alexa 700), for 30 min. Cells were washed twice with 1× Perm Wash buffer and fixed with 250 µl of freshly prepared 1.5% formaldehyde. Fixed cells were transferred to 96-well round bottom plate and analysed by BD FACSymphony system.

Immunophenotyping of BAL cells

BAL cells were stained with Aqua live/dead dye for 20 min, washed with 2% FBS/DPBS buffer, and stained with monoclonal antibodies against CD8 (clone SK1, FITC), CD123 (clone 7G3, PE), CD28 (clone 28.2, PE CF594), CD4 (clone L200, BB700), CD159a (clone Z199, PE CY7), CD49d (clone 9F10, BV421), CD20 (clone 2H7, BV570), CD45 (clone D058-1283, BV605), TCR γδ (clone B1, BV650), CD95 (clone DX2, BV711), CD163 (clone GHI/61, BV786), CD16 (clone 3G8, BUV395), CD14 (clone M5E2, BUV737), CD66 (clone TET2, APC), CD3 (clone SP34.2, Alexa 700), HLA-DR (clone G46-6, APC H7), for 30 min. After staining, cells were washed twice with 2% FBS/DPBS buffer and fixed by 1.5% formaldehyde. All data were acquired on a BD LSRII flow cytometer with FACSDiva software (BD Biosciences). Subsequent analyses were performed using FlowJo software (Treestar, v.9.9.6). For subpopulation quantification, dead cells were excluded by Aqua dye and CD45 was used as a positive inclusion gate for all leukocytes. Lymphocyte populations were quantified using standard rhesus macaque phenotyping protocols and macrophage/granulocyte populations were identified among high granular fractions: macrophages ($CD163^+$); eosinophils ($CD66abce^+CD49d^{hi}$/$modCD14^-$); neutrophils ($CD66abce^+CD49d^{mod}CD14^+$); basophils $CD66abce^-HLA^-DR^-CD123^{hi}$).

Statistical analyses

Analysis of virological and immunologic data was performed using GraphPad Prism 8.4.2 (GraphPad Software). Comparison of data between groups was performed using two-sided Mann–Whitney tests. Correlations were assessed by two-sided Spearman rank-correlation tests. *P* values of less than 0.05 were considered significant.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All data are available in the Article and its Supplementary Information. Source data are provided with this paper.

30. Brown, E. P. et al. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. *J. Immunol. Methods* **443**, 33–44 (2017).
31. Ackerman, M. E. et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. *J. Immunol. Methods* **366**, 8–19 (2011).
32. Lu, L. L. et al. A functional role for antibodies in tuberculosis. *Cell* **167**, 433–443 (2016).
33. Fischinger, S. et al. A high-throughput, bead-based, antigen-specific assay to assess the ability of antibodies to induce complement activation. *J. Immunol. Methods* **473**, 112630 (2019).

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Author contributions D.H.B., R.Z., F.W., P.S., M.M., J.V.H. and H.S. designed the study and reviewed all data. R.Z., F.W., L.R., R.B., D.M., J.V., J.C., J.P.L., T.K., M.J.G.B., D.Z., S.K.R.H., H.S., B.C., J.L., Z.L. and D.H.B. designed the vaccines. N.B.M., A.C., J.Y., J.L., L.P., K.M., L.H.T., E.A.B., G.D., M.S.G., X.H., E.H., C.J.D., M.K., Z.L., S.H.M., L.F.M., F.N., R.N., J.P.N., S.P., J.D.V., K.V., H.W. and R.K.R. performed the immunological and virological assays. C.L., C.A., S.F., J.S.B., D.A.L. and G.A. performed the systems serology. D.R.M. and R.S.B. performed the live virus neutralization assays. L.P., A.V.R., K.B., A.S., M.C., R.B., A.C., S.Z., E.T., H.A. and M.G.L. led the clinical care of the macaques. J.F., B.M.H., T.M.C., Y.C., B.C. and A.G.S. provided purified proteins. D.H.B. wrote the paper with all co-authors.

Competing interests D.H.B., R.Z., F.W., L.R., R.B., D.M., J.V., J.C., J.P.L., T.K., M.J.G.B. and H.S are co-inventors on provisional vaccine patents (62/969,008; 62/994,630). R.Z., F.W., L.R., R.B., D.M., J.V., J.C., J.P.L., T.K., M.J.G.B., D.Z., S.K.R.H., P.S., M.M., J.V.H. and H.S. are employees of Janssen Vaccines and Prevention BV and hold stock in Johnson and Johnson.

Additional information

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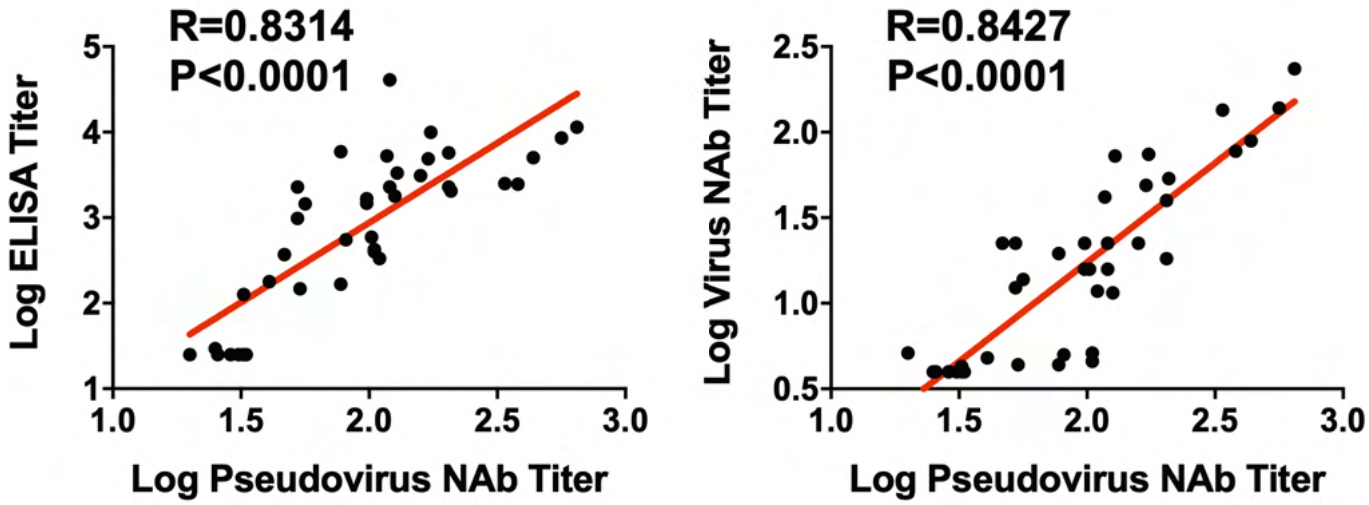
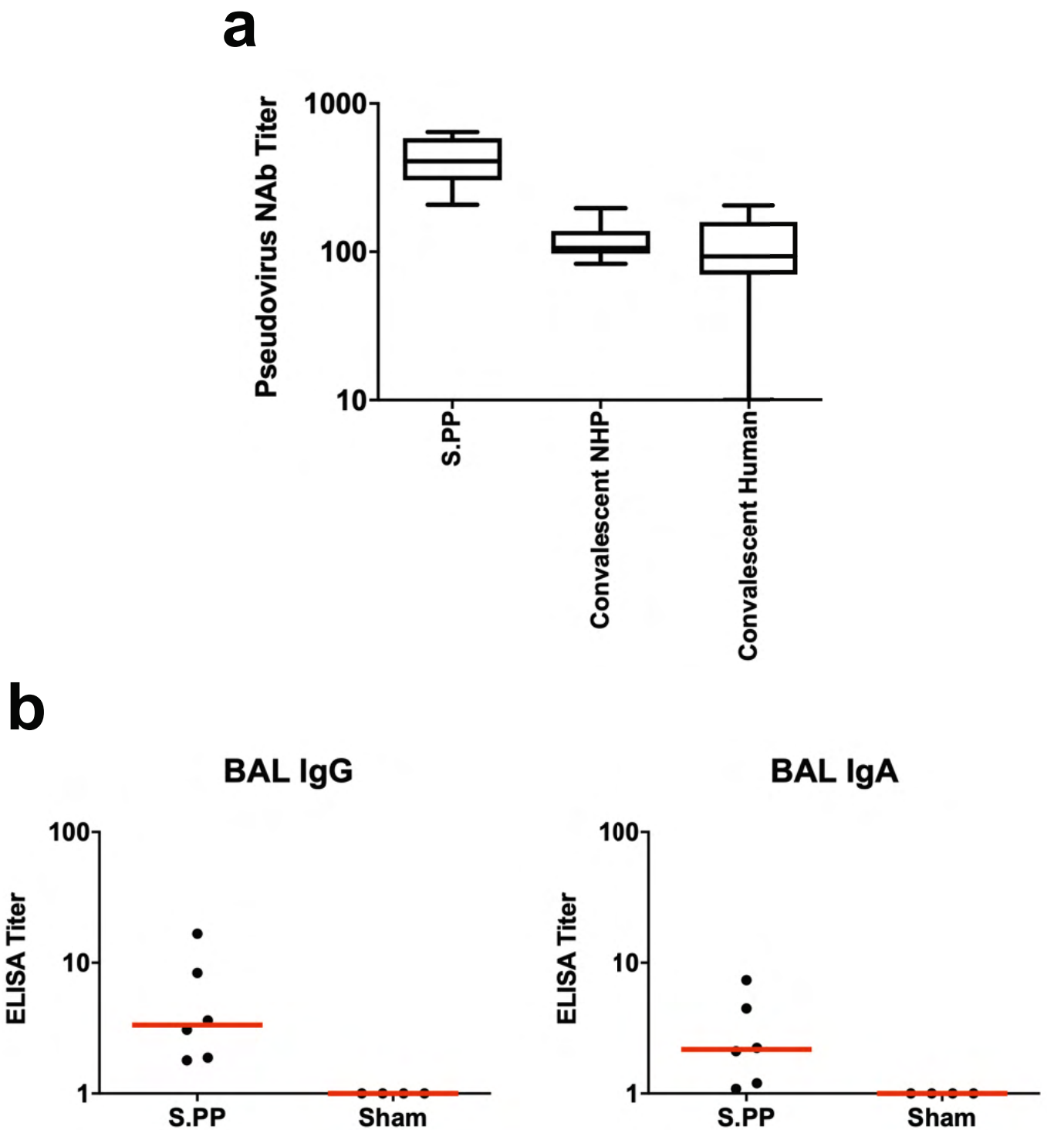
Correspondence and requests for materials should be addressed to D.H.B.

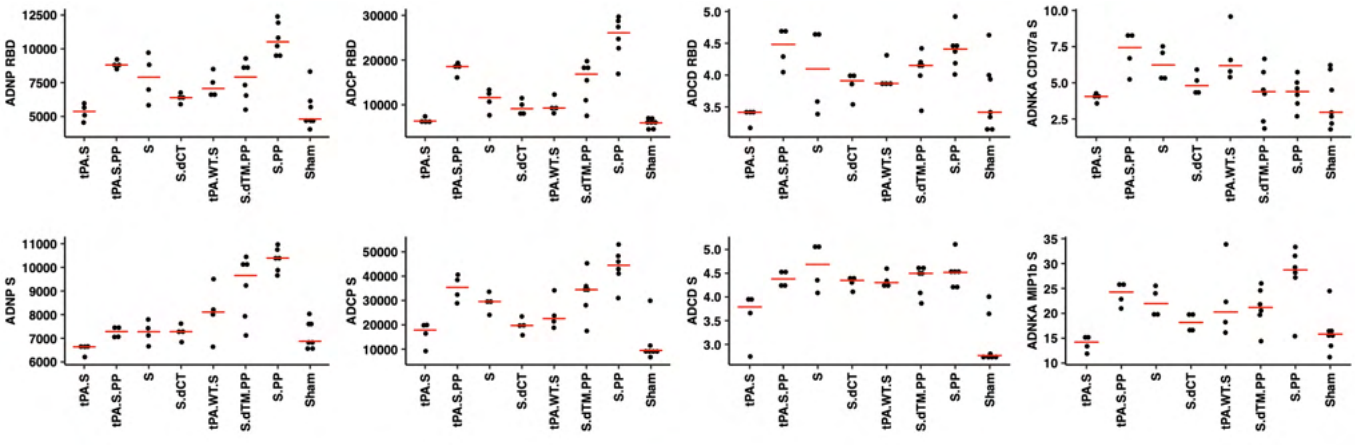
Peer review information *Nature* thanks Wolfgang Baumgärtner, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

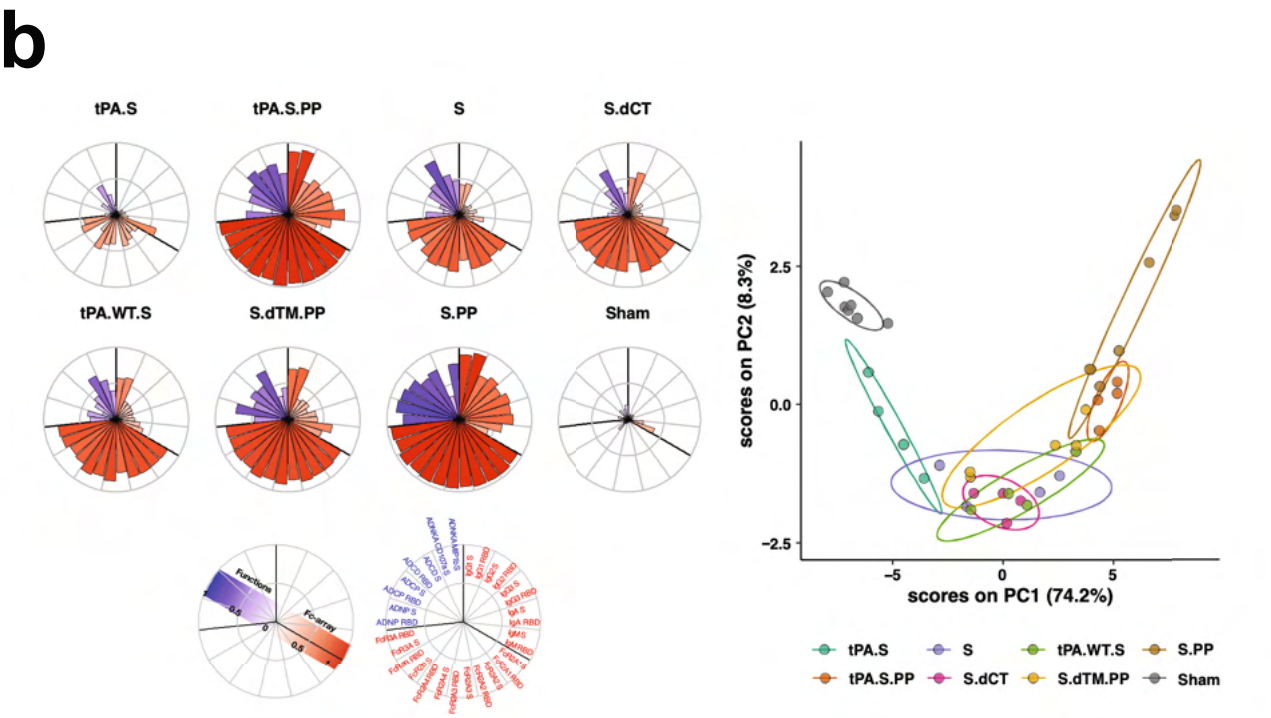


Extended Data Fig. 1 | Correlation of pseudovirus neutralizing antibody titres and ELISA or live virus neutralizing antibody assays in vaccinated macaques. Red line reflects the best linear fit relationship between these variables. *P* and *R* values reflect two-sided Spearman rank-correlation tests. *n* = 38 biologically independent macaques.

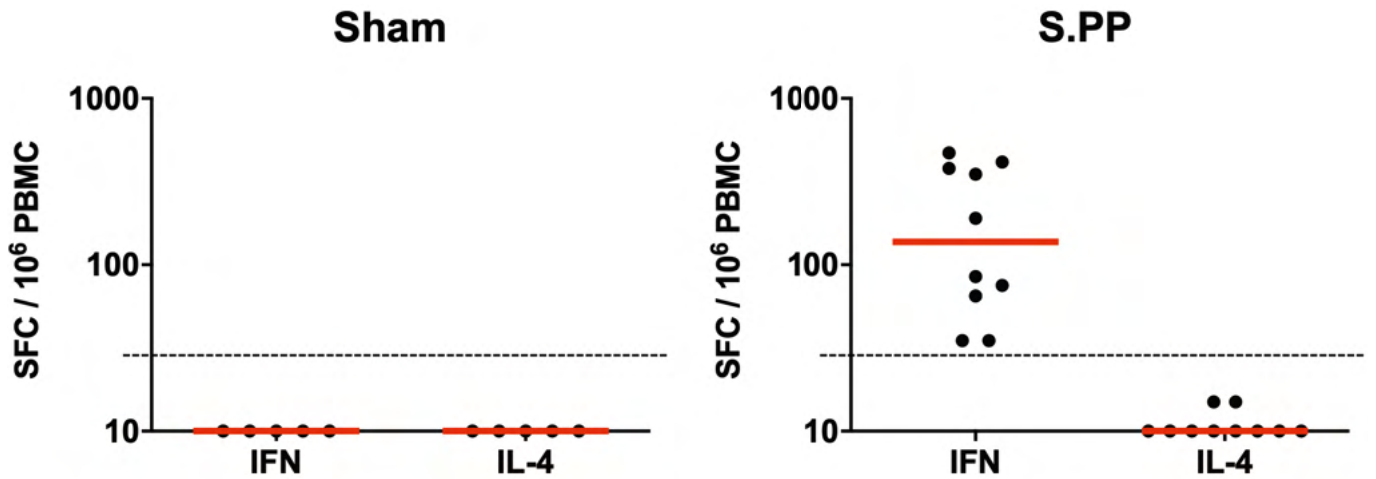


Extended Data Fig. 2 | Peripheral and mucosal humoral immune responses in vaccinated rhesus macaques. a, Comparison of pseudovirus neutralizing antibodies in macaques vaccinated with Ad26-S.PP ($n = 6$ biologically independent macaques) with previously reported cohorts of convalescent macaques[9] ($n = 9$ biologically independent macaques) and convalescent humans[10] ($n = 27$ biologically independent humans) who had recovered from SARS-CoV-2 infection. NHP, non-human primate. The upper bound of the box is the seventy-fifth and the lower bound the twenty-fifth percentile, the horizontal line indicates the median and the whiskers extend from the box bounds to the minimum/maximum value. **b**, S-specific IgG and IgA at week 4 in BAL by ELISA in sham controls and in Ad26-S.PP vaccinated macaques. Red bars reflect median responses.

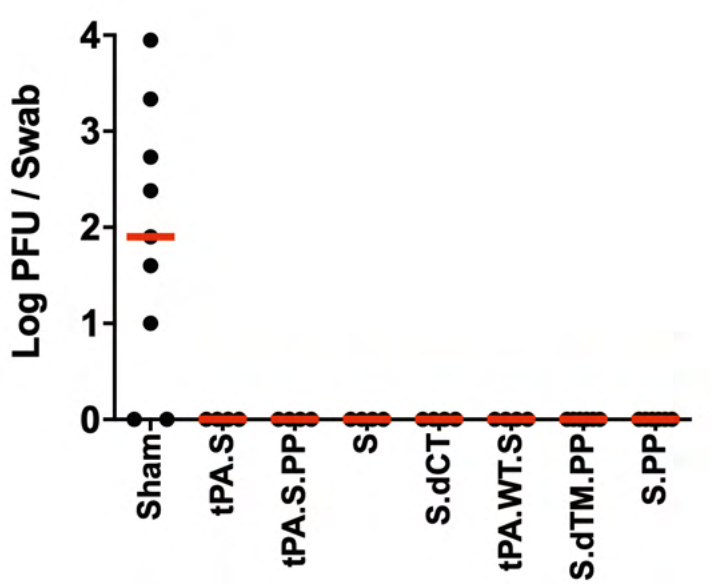




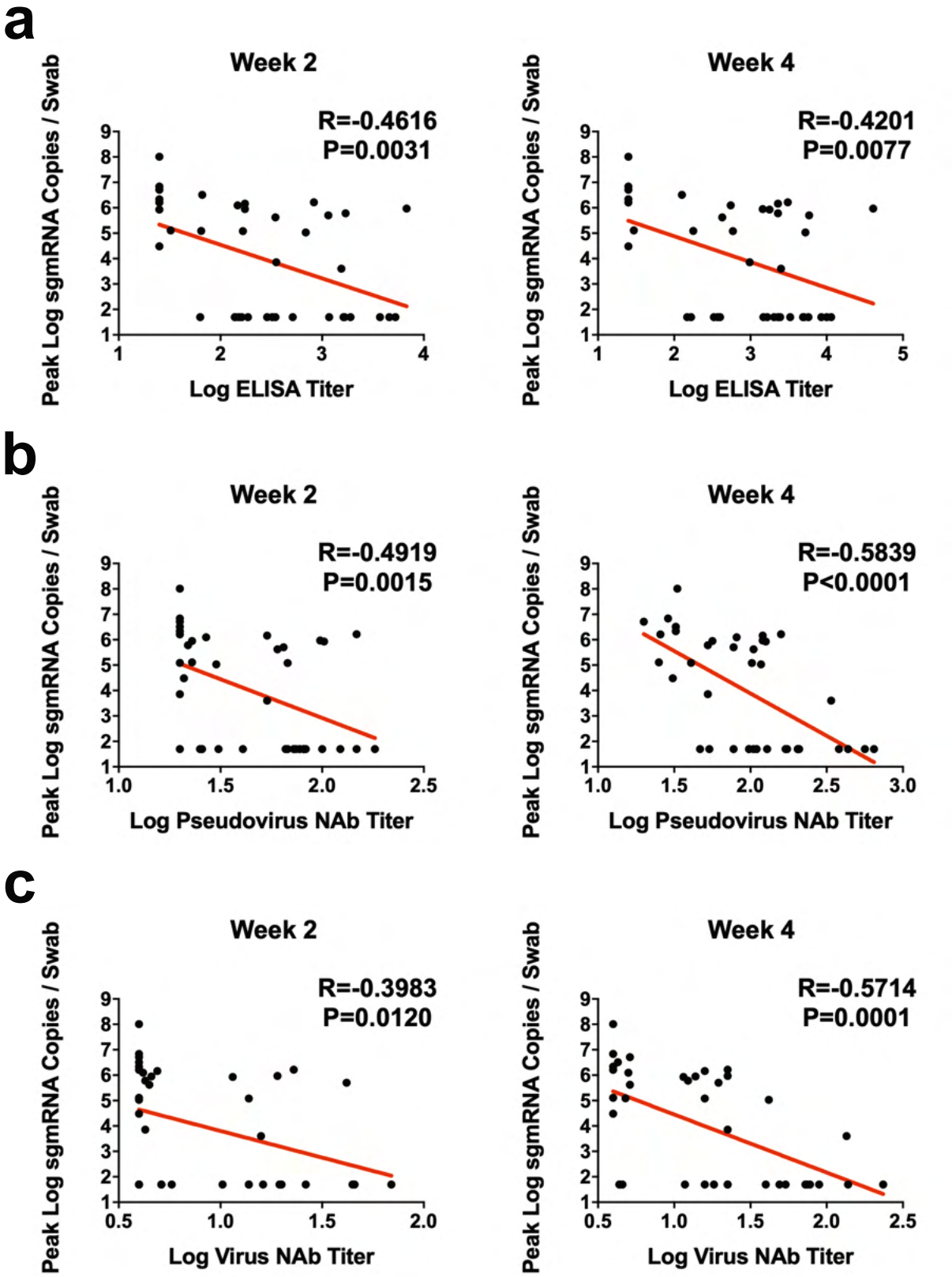
Extended Data Fig. 3 | Systems serology in vaccinated rhesus macaques. **a**, S-specific and RBD-specific ADNP, ADCP, ADCD and ADNKA assays are shown. Red bars reflect median responses. **b**, S-specific and RBD-specific ADNP, ADCP, ADCD and ADNKA assays at week 4 are shown as radar plots. The size and colour intensity of the wedges indicate the median of the feature for the corresponding group (blue depicts antibody functions; red depicts antibody isotype, subclass or FcγR binding). The principal component analysis (PCA) plot shows the multivariate antibody profiles across groups. The PCA analysis reduces the dimension of the data by finding principal components, which are linear combinations of the original antibody features that are uncorrelated and best capture the variance in the data. Here, PC1 explains 74.2% and PC2 explains 8.3% of the variance. Each dot represents a macaque, the colour of the dot denotes the group, and the ellipses shows the distribution of the groups as 70% confidence levels assuming a multivariate normal distribution.



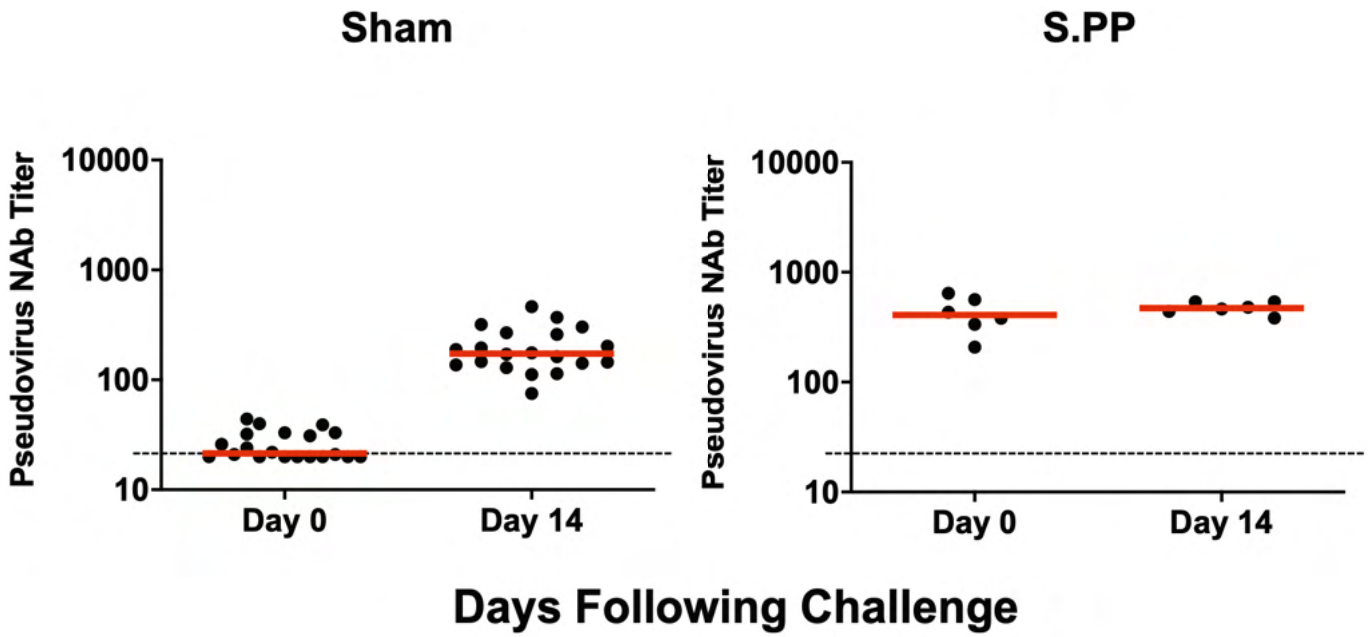
Extended Data Fig. 4 | Cellular immune responses in vaccinated rhesus macaques. IFNγ and IL-4 ELISPOT responses in response to pooled S peptides were assessed in a separate cohort of 10 macaques that received 1×10^{11} viral particles of the Ad26-S.PP vaccine at week 2 after vaccination. Red bars reflect median responses. Dotted lines reflect assay limit of quantification.



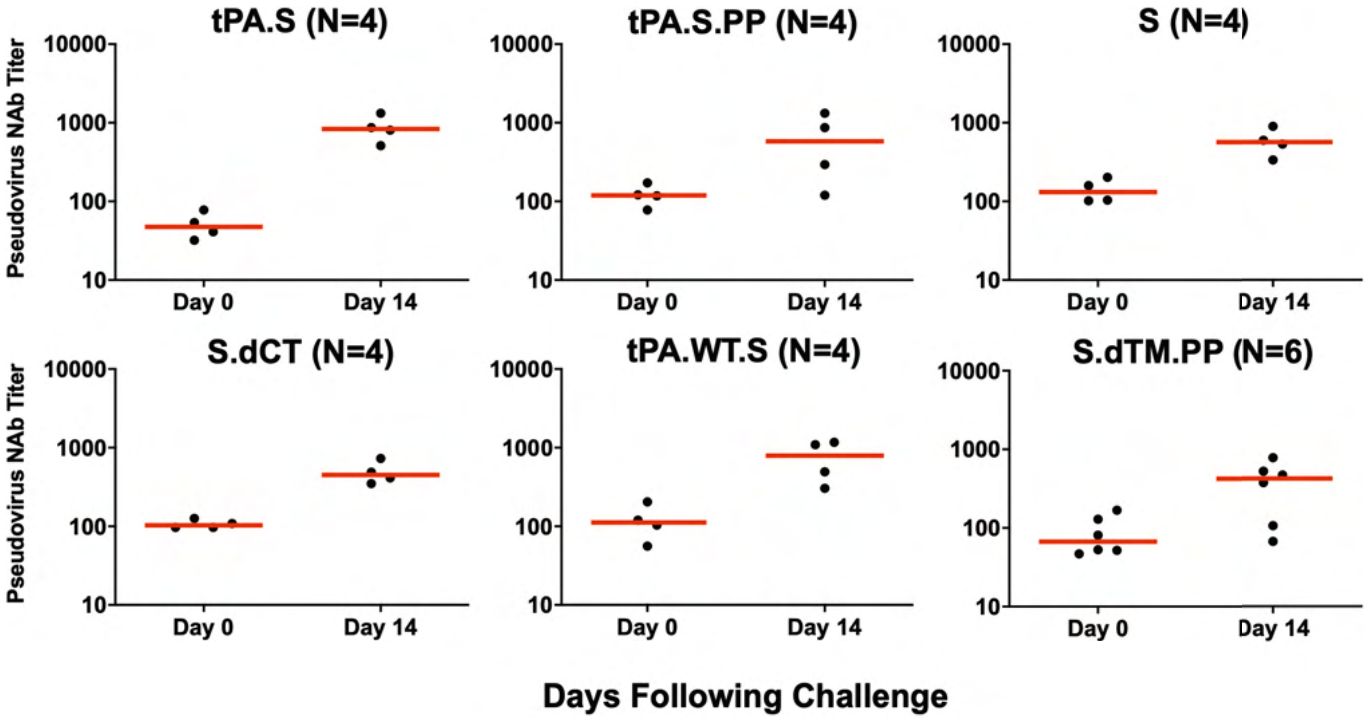
Extended Data Fig. 5 | Infectious virus after challenge. Infectious virus titres were assessed by PFU assays in nasal swabs on day 2 after challenge in vaccinated macaques and additional sham controls.



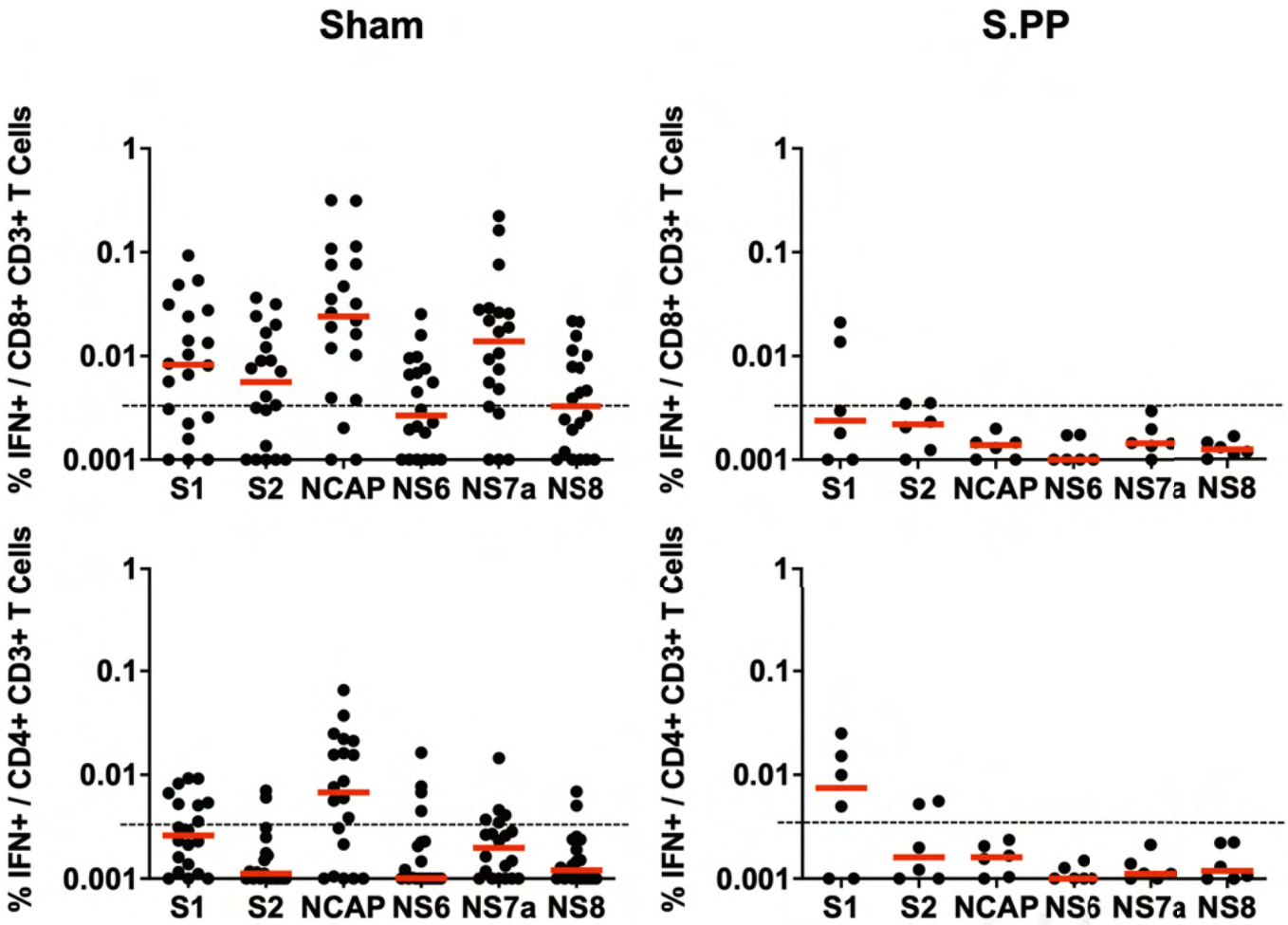
Extended Data Fig. 6 | Correlates of protection in nasal swabs. **a**–**c**, Correlations of binding ELISA titres (**a**), pseudovirus neutralizing antibody titres (**b**), and (**c**) live virus neutralizing antibody titres at weeks 2 and 4 with log[peak sgRNA (copies per swab)] in nasal swabs after challenge. Red lines reflect the best linear fit relationship between these variables. *P* and *R* values reflect two-sided Spearman rank-correlation tests. $n = 52$ biologically independent macaques.



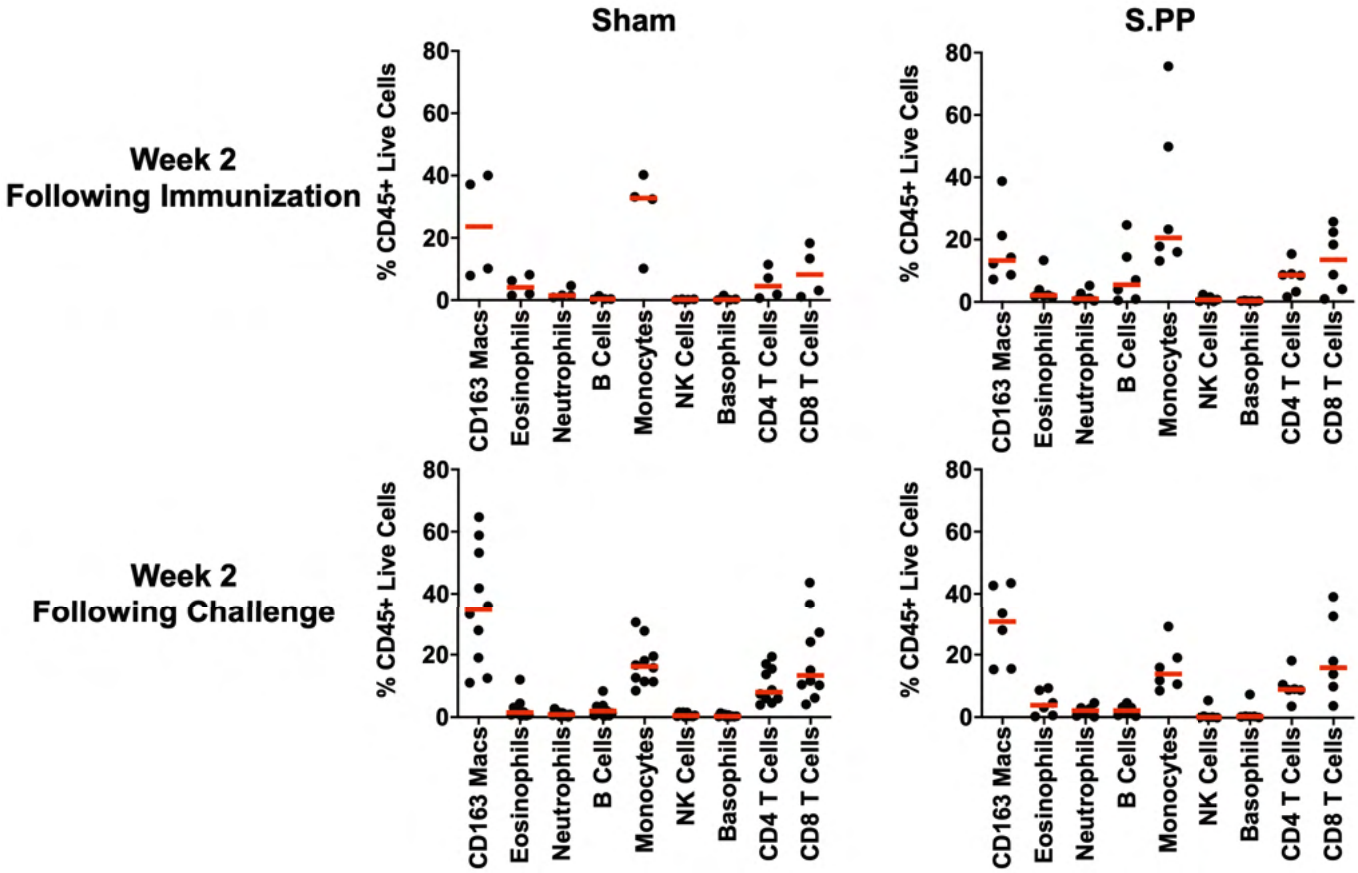
Extended Data Fig. 7 | Neutralizing antibody titres after SARS-CoV-2 challenge. Pseudovirus neutralizing antibody titres before challenge and on day 14 after challenge in sham controls and in Ad26-S.PP vaccinated macaques. Red bars reflect median responses. Dotted lines reflect assay limit of quantification.



Extended Data Fig. 8 | Neutralizing antibody titres after SARS-CoV-2 challenge. Pseudovirus neutralizing antibody titres before challenge and on day 14 after challenge in vaccinated macaques. Red bars reflect median responses. Dotted lines reflect assay limit of quantification.



Extended Data Fig. 9 | Cellular immune responses after SARS-CoV-2 challenge. IFNγ$^+$CD8$^+$ and IFNγ$^+$CD4$^+$ T cell responses by intracellular cytokine staining assays in response to pooled spike (S1 and S2), nucleocapsid (NCAP), and non-structural proteins (N6, N7a and N8) peptides on day 14 after challenge in sham controls and in Ad26-S.PP-vaccinated macaques. Red bars reflect median responses. Dotted lines reflect assay limit of quantification.



Extended Data Fig. 10 | Immunophenotyping of BAL cell subpopulations. BAL cells from Ad26-S.PP-vaccinated and control macaques from 2 weeks after immunization and 2 weeks after challenge were assessed by flow cytometry for cellular subpopulations.

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☐	☒ Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect data.
Data analysis	Analysis of virologic and immunologic data was performed using R and GraphPad Prism 8.4.2 (GraphPad Software).

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- A list of figures that have associated raw data
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All data are available in the manuscript or the supplementary material. Correspondence and requests for materials should be addressed to D.H.B. (dbarouch@bidmc.harvard.edu).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size includes N=32 vaccinated animals (N=4-6 animals for each vaccine group; Yu et al Science 2020) and N=20 sham controls. Based on our experience with SARS-CoV-2 in rhesus macaques, this sample size provides power to determine differences in protective efficacy of each vaccinated group compared with the sham controls.
Data exclusions	No data were excluded. One animal in the S.dTM.PP group did not have peak BAL samples obtained following challenge for veterinary reasons (described in Figure 4 legend).
Replication	Virologic and immunologic measures were performed in duplicate. Technical replicates were minimally different.
Randomization	Animals were balanced for age and gender and otherwise randomly allocated to groups.
Blinding	All immunologic and virologic assays were performed blinded.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	SARS-CoV-2 virus stocks are available from BEI. Other reagents can be shared with an MTA for academic research.

Antibodies

Antibodies used	For ELISA and ELISPOT assays anti-macaque IgG HRP (NIH NHP Reagent Program), rabbit polyclonal anti-human IFN-γ (U-Cytech); for ICS assays mAbs against CD279 (clone EH12.1, BB700), CD38 (clone OKT10, PE), CD28 (clone 28.2, PE CY5), CD4 (clone L200, BV510), CD45 (clone D058-1283, BUV615), CD95 (clone DX2, BUV737), CD8 (clone SK1, BUV805), Ki67 (clone B56, FITC), CD69 (clone TP1.55.3, ECD), IL10 (clone JES3-9D7, PE CY7), IL13 (clone JES10-5A2, BV421), TNF-α (clone Mab11, BV650), IL4 (clone MP4-25D2, BV711), IFN-γ (clone B27; BUV395), IL2 (clone MQ1-17H12, APC), CD3 (clone SP34.2, Alexa 700) (BD); for BAL cell immunophenotyping mAbs against CD8 (clone SK1, FITC), CD123 (clone 7G3, PE), CD28 (clone 28.2, PE CF594), CD4 (clone L200, BB700), CD159a (clone Z199, PE CY7), CD49d (clone 9F10, BV421), CD20 (clone 2H7, BV570), CD45 (clone D058-1283, BV605), TCR γδ (clone B1, BV650), CD95 (clone DX2, BV711), CD163 (clone GHI/61, BV786), CD16 (clone 3G8, BUV395), CD14 (clone M5E2, BUV737), CD66 (clone TET2, APC), CD3 (clone SP34.2, Alexa 700), HLA-DR (clone G46-6, APC H7); CR3046 human monoclonal antibody (Janssen); 800CW-conjugated goat-anti-human secondary antibody (Li-COR); anti-rhesus IgG1, IgG2, IgG3, IgA, IgM (NIH NHP Reagent Program); tertiary goat anti-mouse IgG-PE antibody (Southern Biotech), anti-CD107a (PE-Cy7, BD), anti-CD56 (PE-Cy7, BD), anti-MIP-1β (PE, BD), mouse anti-human IFN-γ monoclonal antibody (BD), Streptavidin-alkaline phosphatase antibody (Southern Biotech).

Validation	all mAbs used according to manufacturer's instructions and previously published methods; mAbs were validated and titrated for specificity prior to use

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero E6, HEK293T, THP-1 cells, MRC-5 cells
Authentication	Commerically purchased (ATCC)
Mycoplasma contamination	Negative for mycoplasma
Commonly misidentified lines (See ICLAC register)	N/A

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	52 outbred Indian-origin adult male and female rhesus macaques (Macaca mulatta), 6-12 years old
Wild animals	None
Field-collected samples	None

Human research participants

Policy information about studies involving human research participants

Population characteristics	De-identified human PBMC were commercially purchased; no studies involved human research participants
Recruitment	None

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Ad26 vaccine protects against SARS-CoV-2 severe clinical disease in hamsters

Lisa H. Tostanoski[1,10], Frank Wegmann[2,10], Amanda J. Martinot[1,3,10], Carolin Loos[4,5,10], Katherine McMahan[1,10], Noe B. Mercado[1,10], Jingyou Yu[1,10], Chi N. Chan[6], Stephen Bondoc[6], Carly E. Starke[6], Michael Nekorchuk[6], Kathleen Busman-Sahay[6], Cesar Piedra-Mora[1,3], Linda M. Wrijil[3], Sarah Ducat[3], Jerome Custers[2], Caroline Atyeo[4,7], Stephanie Fischinger[4,7], John S. Burke[4], Jared Feldman[4,7], Blake M. Hauser[4,7], Timothy M. Caradonna[4,7], Esther A. Bondzie[1], Gabriel Dagotto[1,7], Makda S. Gebre[1,7], Catherine Jacob-Dolan[1,7], Zijin Lin[1], Shant H. Mahrokhian[1], Felix Nampanya[1], Ramya Nityanandam[1], Laurent Pessaint[8], Maciel Porto[8], Vaneesha Ali[8], Dalia Benetiene[8], Komlan Tevi[8], Hanne Andersen[8], Mark G. Lewis[8], Aaron G. Schmidt[4,7,9], Douglas A. Lauffenburger[5], Galit Alter[4,9], Jacob D. Estes[6], Hanneke Schuitemaker[2], Roland Zahn[2] and Dan H. Barouch[1,4,7,9] ✉

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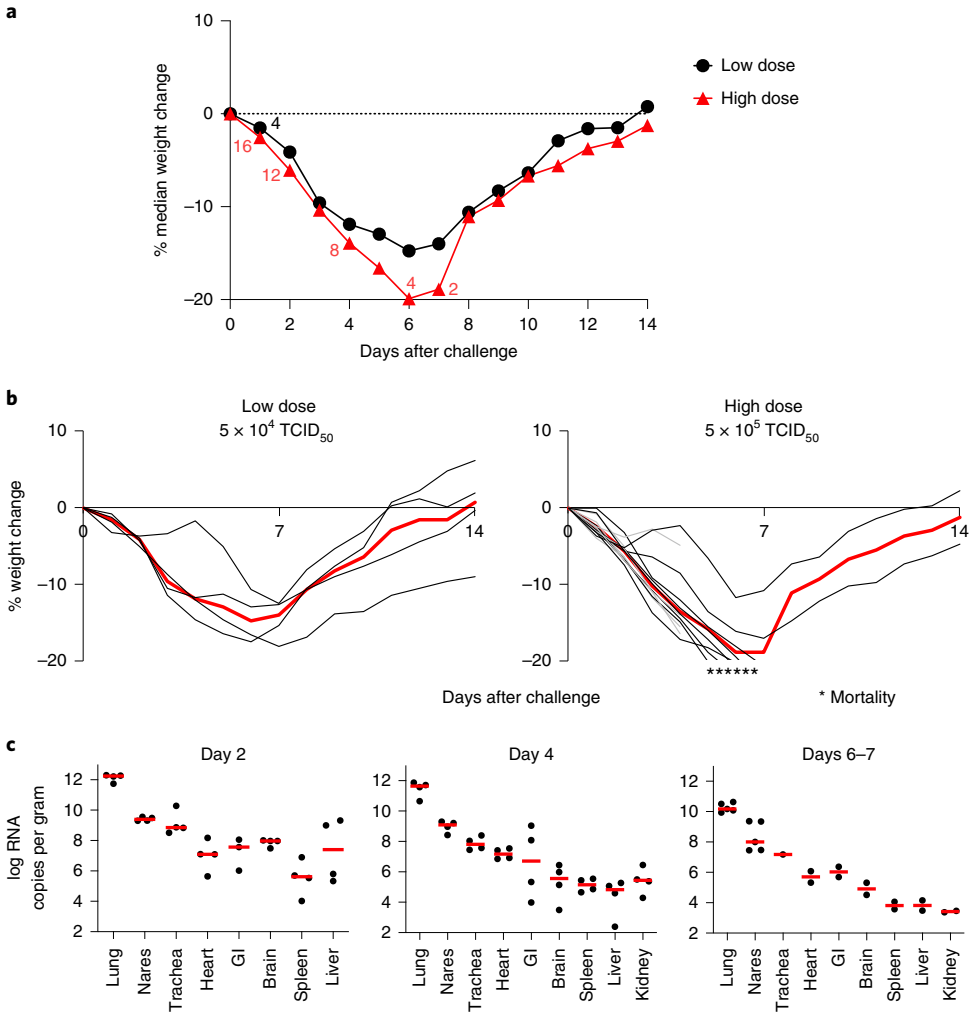
SARS-CoV-2 can infect nonhuman primates[8–10], hamsters[5–7], ferrets[14–16], hACE2 transgenic mice[17,18] and other species[16], but clinical disease in these models has generally been mild. A severe pneumonia model would be useful for preclinical evaluation of SARS-CoV-2 vaccines and other countermeasures, because SARS-CoV-2 infection in humans can lead to severe clinical disease, respiratory failure and mortality[1–4]. We assessed the clinical and virologic characteristics of high-dose SARS-CoV-2 infection in hamsters and evaluated the protective efficacy of an adenovirus serotype 26 (Ad26) vector-based vaccine[19] encoding a stabilized SARS-CoV-2 spike (S) in this stringent model.

We inoculated 20 Syrian golden hamsters (10–12 weeks old) with 5×10^4 50% tissue culture infective dose ($TCID_{50}$) ($n=4$; low-dose) or 5×10^5 $TCID_{50}$ ($n=16$; high-dose) SARS-CoV-2 by the intranasal route. In the high-dose group, four animals were necropsied on day 2, four animals were necropsied on day 4 for tissue viral loads and histopathology and the remaining eight animals were followed longitudinally. All remaining animals were necropsied on day 14. In the low-dose group, hamsters lost a median of 14.7% of body weight by day 6 but fully recovered by day 14 (Fig. 1a,b), consistent with previous studies[5–7]. In the high-dose group, hamsters lost a median of 19.9% of body weight by day 6. Of the eight animals in this group that were followed longitudinally, four met Institutional Animal Care and Use Committee humane euthanasia criteria of more than 20% weight loss and respiratory distress on day 6, and two additional animals met these criteria on day 7. The remaining two animals recovered by day 14. These data demonstrate that high-dose SARS-CoV-2 infection in hamsters led to severe weight loss and partial mortality.

Tissue viral loads were assessed in the four animals that received high-dose SARS-CoV-2 and were necropsied on day 2, the four animals that were necropsied on day 4 and five of six of the animals that met euthanasia criteria on days 6–7 (Fig. 1c). High median tissue viral loads on day 2 of 10^{12} RNA copies per gram in lung tissue and 10^8–10^9 RNA copies per gram in nares and trachea were observed, with a median of 10^5–10^8 RNA copies per gram in heart, gastrointestinal tract, brain, spleen, liver and kidney, indicative of disseminated infection. By days 6–7, tissue viral loads were approximately 2 logs lower, despite continued weight loss.

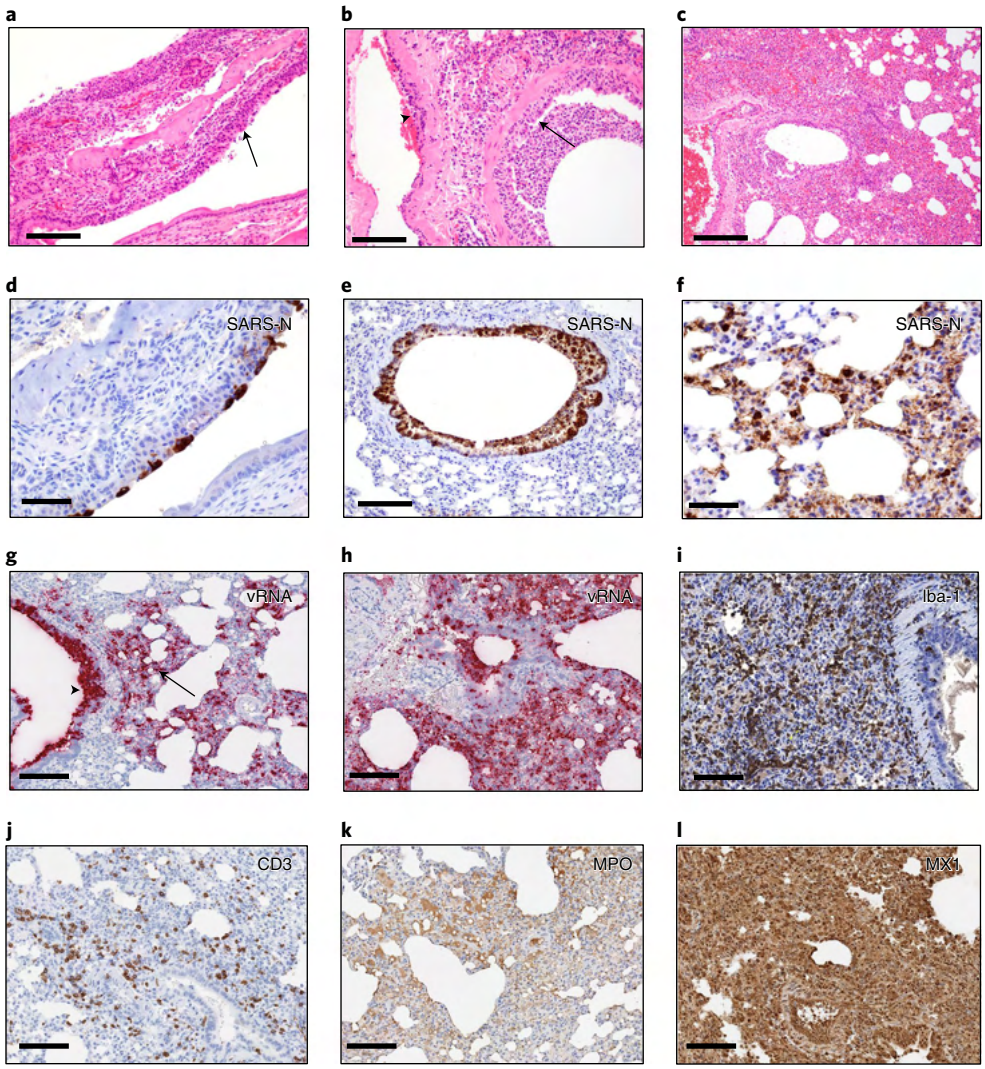
[1]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA. [2]Janssen Vaccines & Prevention BV, Leiden, Netherlands. [3]Tufts University Cummings School of Veterinary Medicine, North Grafton, MA, USA. [4]Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, USA. [5]Massachusetts Institute of Technology, Cambridge, MA, USA. [6]Oregon Health & Sciences University, Beaverton, OR, USA. [7]Harvard Medical School, Boston, MA, USA. [8]Bioqual, Rockville, MD, USA. [9]Massachusetts Consortium on Pathogen Readiness, Boston, MA, USA. [10]These authors contributed equally: Lisa H. Tostanoski, Frank Wegmann, Amanda J. Martinot, Carolin Loos, Katherine McMahan, Noe B. Mercado, Jingyou Yu. ✉e-mail: dbarouch@bidmc.harvard.edu



Clinical disease after SARS-CoV-2 infection in hamsters. Syrian golden hamsters (10–12 weeks old; male and female; $n=20$) were infected with 5×10^4 $TCID_{50}$ (low-dose; $n=4$) or 5×10^5 $TCID_{50}$ (high-dose; $n=16$) of SARS-CoV-2 by the intranasal route. , Median percent weight change after challenge. The numbers reflect the number of animals at each time point. In the high-dose group, four animals were necropsied on day 2, four animals were necropsied on day 4, four animals met euthanization criteria on day 6 and two animals met euthanization criteria on day 7. , Percent weight change after challenge in individual animals. Median weight loss is depicted in red. Asterisks indicate mortality. Gray lines indicate animals with scheduled necropsies on day 2 and day 4. , Tissue viral loads as measured by log_{10} RNA copies per gram of tissue (limit of quantification, 100 copies per gram) in the scheduled necropsies at day 2 and day 4 and in 2–5 of 6 animals that met euthanization criteria on days 6–7. Extended tissues were not harvested on day 6.

Hamsters infected with high-dose SARS-CoV-2 were assessed by histopathology on days 2 ($n=4$), 4 ($n=4$), 6–7 ($n=6$) and 14 ($n=2$). Infection was associated with marked inflammatory infiltrates and multifocal epithelial necrosis of the nasal turbinate (Fig. 2a) and bronchiolar epithelium, resulting in degenerative neutrophils and cellular debris in the lumen (Fig. 2b). The endothelium of nearby vessels was reactive with adherence of mononuclear cells to the endothelium and transmigrating within vessel walls, indicative of endothelialitis (Fig. 2b). There was moderate to severe multifocal interstitial pneumonia characterized by pulmonary consolidation affecting 30–60% of the lung parenchyma as early as day 2 after SARS-CoV-2 infection (Fig. 2c). Inflammatory infiltrates consisted of massive numbers of macrophages and neutrophils with fewer lymphocytes. The nasal turbinate epithelium (Fig. 2d) and bronchiolar epithelial cells (Fig. 2e) were strongly positive for SARS nucleocapsid protein (SARS-CoV-N) by immunohistochemistry (IHC) in regions of inflammation and necrosis. SARS-CoV-N IHC also showed locally extensive staining of the alveolar septa and interstitial mononuclear cells morphologically consistent with macrophages (Fig. 2f). Similarly, substantial SARS-CoV-2 viral RNA (vRNA) was observed in the bronchiolar epithelium and the pulmonary interstitium in regions of inflammation (Fig. 2g,h).

Levels of both SARS-CoV-2 vRNA and SARS-CoV-N protein expression in lung were highest on day 2 and diminished by day 4, with minimal vRNA and SARS-CoV-N protein detected by day 7 (Extended Data Fig. 1). The pneumonia was characterized by large inflammatory infiltrates of ionized calcium-binding adaptor protein 1 (Iba-1)$^+$ macrophages in the lung interstitium as well as $CD3^+$ T lymphocytes (Fig. 2i,j). Many viable and degenerative neutrophils were detected throughout the lung, especially in regions of necrosis, with high expression of neutrophil myeloperoxidase (MPO) throughout the lung (Fig. 2k). Diffuse expression of the interferon inducible gene product MX1 was also detected in the lung (Fig. 2l). In contrast with the kinetics of SARS-CoV-2 vRNA and SARS-CoV-N detection, which peaked on day 2, these markers of inflammation peaked on day 7 (Extended Data Fig. 1), coincident with maximal weight loss and mortality (Fig. 1a,b). Detection of vRNA in the lung by RNAscope did not simply reflect the viral inoculum, as we detected not only negative



Pathologic features of high-dose SARS-CoV-2 infection in hamsters. , Necrosis and inflammation (arrow) in nasal turbinate, H&E (day 2). , Bronchiolar epithelial necrosis with cellular debris and degenerative neutrophils in lumen (arrow) and transmigration of inflammatory cells in vessel wall (arrowhead), H&E (day 2). , Interstitial pneumonia, hemorrhage and consolidation of lung parenchyma, H&E (day 2). , Nasal turbinate epithelium shows strong positivity for SARS-CoV-N by IHC (day 2). , Bronchiolar epithelium and luminal cellular debris show strong positivity for SARS-CoV-N by IHC (day 2). Pneumocytes and alveolar septa show multifocal strong positivity for SARS-CoV-N by IHC (day 2). , Diffuse vRNA staining by RNAscope within pulmonary interstitium (arrow, interstitial pneumonia) and within bronchiolar epithelium (arrowhead; day 2). , Diffuse vRNA staining by RNAscope within pulmonary interstitium (day 4). Iba-1 IHC (macrophages) within pulmonary interstitium (day 7). $CD3^+$ T lymphocytes within pulmonary interstitium, CD3 IHC (day 4). , MPO IHC indicating presence of interstitial neutrophils (day 7). Interferon inducible gene MX1 IHC shows strong and diffuse positivity throughout the lung (day 4). Representative sections are shown. Experiments were repeated at least three times with similar results. Scale bars, 20 μm (,); 50 μm (, ,); and 100 μm (– . H&E, hematoxylin and eosin.

anti-sense vRNA (Extended Data Fig. 2a–e) but also positive-sense vRNA (Extended Data Fig. 2f–j), which overlapped in location and pattern, from day 2 to day 7 after challenge. SARS-CoV-2 vRNA expression (both anti-sense and sense) was present in lung with robust ACE2 receptor expression (Extended Data Fig. 2k–o).

Systemic vRNA was also detected in distal tissues, including the brain stem, gastrointestinal tract and myocardium (Extended Data Fig. 3a–f). Prominent endothelialitis and perivascular inflammation with macrophages and lymphocytes was observed in these tissues, despite minimal SARS-CoV-N staining (Extended Data Fig. 3g–j). Focal lymphocytic myocarditis was noted in one animal and corresponded to the presence of vRNA (Extended Data Fig. 3k–l). Other sites of virus detection included peripheral blood mononuclear cells in thrombi in lung (Extended Data Fig. 4a–c) and bone marrow of the nasal turbinate (Extended Data Fig. 4d–f).

We produced recombinant, replication-incompetent Ad26 vectors encoding 1) SARS-CoV-2 S with deletion of the transmembrane region and cytoplasmic tail reflecting the soluble ectodomain with a foldon trimerization domain (S.dTM.PP) or 2) full-length S (S.PP), both with mutation of the furin cleavage site and two proline stabilizing mutations[20] (Fig. 3a). We recently reported the immunogenicity and protective efficacy of these vaccines against SARS-CoV-2 challenge in rhesus macaques[13].

We immunized 50 Syrian golden hamsters with 10^{10} or 10^9 viral particles (vp) of Ad26 vectors encoding S.dTM.PP or S.PP ($n=10$ per group) or sham controls ($n=10$). Animals received a single vaccination by the intramuscular route at week 0. We observed receptor-binding domain (RBD)-specific binding antibodies by enzyme-linked immunosorbent assay (ELISA)[10,11] (Fig. 3b) and neutralizing antibodies (NAbs) by a pseudovirus neutralization

assay[10,11,21] (Fig. 3c) in all animals at week 2 and week 4. At week 4, Ad26-S.PP elicited 4.0–4.7-fold higher median ELISA titers (4,470, 4,757) compared to Ad26-S.dTM.PP (1,014, 1,185) (Fig. 3b; $P<0.0001$, two-sided Mann–Whitney tests). Similarly, Ad26-S.PP elicited 1.8–2.6-fold higher median NAb IC_{50} titers (359, 375) compared to Ad26-S.dTM.PP (139, 211) ($P<0.05$, two-sided Mann–Whitney tests). For each vector, the two doses tested appeared to be similar. ELISA and NAb data were correlated at both week 2 and week 4 ($R=0.7074$, $P<0.0001$ and $R=0.7849$, $P<0.0001$, respectively, two-sided Spearman's rank correlation tests; Extended Data Fig. 5a).

We further characterized S-specific and RBD-specific antibody responses in the vaccinated animals at week 4 by systems serology[22]. IgG, IgG2a, IgG3, IgM, Fc-receptors FcRγ2, FcRγ3 and FcRγ4 and antibody-dependent complement deposition (ADCD) responses were assessed (Fig. 3d–f). Higher and more consistent responses were observed with Ad26-S.PP than with Ad26.S.dTM.PP (Fig. 3d,f), and a principal component analysis (PCA) of these antibody features confirmed that these two vaccines had distinct profiles (Fig. 3e).

At week 4, all animals were challenged with 5×10^5 $TCID_{50}$ SARS-CoV-2 by the intranasal route. Three animals in each group were necropsied on day 4 for tissue viral loads and histopathology, and the remaining seven animals in each group were followed until day 14. In the sham controls, hamsters lost a median of 19.6% of body weight by day 7, and 43% (3/7) of the animals that were followed longitudinally met euthanasia criteria on days 6–7 (Fig. 4a,b). The Ad26-S.dTM.PP vaccinated animals lost a median of 8.7% body weight, and the Ad26-S.PP vaccinated animals lost a median of 4.0% body weight (Fig. 4a,b). Maximum percent weight loss was markedly lower in both vaccinated groups compared to sham controls ($P<0.0001$, two-sided Mann–Whitney tests; Fig. 4c), and animals that received Ad26-S.PP showed less weight loss than animals that received Ad26.S.dTM.PP ($P < 0.0001$, two-sided Mann–Whitney tests; Fig. 4c). Both vaccines protected against mortality, defined as meeting humane euthanization criteria, as compared to sham controls ($P=0.02$, two-sided Fisher's exact tests; Extended Data Fig. 5b). A combined analysis of the two hamster experiments confirmed that both vaccines effectively protected against mortality ($P=0.007$, two-sided Fisher's exact tests; Extended Data Fig. 5c). ELISA responses at week 2 ($R=-0.8992$, $P<0.0001$) and week 4 ($R=-0.9344$, $P<0.0001$) correlated inversely with maximum percent weight loss (Extended Data Fig. 6a). NAb responses at week 2 ($R=-0.7380$, $P<0.0001$) and week 4 ($R=-0.8075$, $P<0.0001$) also correlated inversely with maximum percent weight loss (Extended Data Fig. 6b).

Tissue viral loads were assessed in the subset of animals necropsied on day 4 and in the remaining surviving animals on day 14. On day 4 after high-dose SARS-CoV-2 challenge, virus was detected in tissues in all animals by subgenomic RNA reverse trancription–polymerase chain reaction (RT–PCR), which is thought to measure replicating virus[10,23] (Extended Data Fig. 7a). Median viral loads in lung tissue were approximately 10^{12} RNA copies per gram in the sham controls compared to 10^8 RNA copies per gram in the Ad26-S.dTM.PP vaccinated animals and 10^6 RNA copies per gram in the Ad26-S.PP vaccinated animals. Reduced $TCID_{50}$ infectious virus titers per gram of lung tissue were also observed for the Ad26-S.dTM.PP and Ad26.S.PP vaccinated animals compared to sham controls ($P=0.02$ and $P=0.01$, respectively, two-sided Mann–Whitney tests; Extended Data Fig. 7b). By day 14, virus was still detected in lung and nares of the surviving sham controls but was observed in only a minority of Ad26-S.dTM.PP vaccinated animals and in none of the Ad26-S.PP vaccinated animals (Extended Data Fig. 7c).

ELISA responses at week 2 ($R=-0.8133$, $P=0.0004$) and week 4 ($R=-0.9288$, $P<0.0001$) correlated inversely with lung viral loads at day 4 (Extended Data Fig. 8a), and NAb responses at week 2 ($R=-0.7469$, $P=0.0020$) and week 4 ($R=-0.6004$, $P=0.0199$) correlated inversely with lung viral loads at day 4 (Extended Data Fig. 8b). ELISA and NAb responses also correlated inversely with viral loads in nasal turbinates (Extended Data Fig. 8c,d). A deeper analysis of immune correlates revealed that multiple antibody characteristics correlated inversely with weight loss and tissue viral loads (Extended Data Fig. 9a).

The surviving sham controls developed potent binding and neutralizing antibody responses by day 14 after challenge (Extended Data Fig. 9b). Vaccinated animals also demonstrated higher ELISA and NAb responses after challenge (Extended Data Fig. 9b), consistent with tissue viral loads showing low and transient levels of virus replication in these animals after high-dose SARS-CoV-2 challenge.

Vaccinated animals also demonstrated diminished pathology compared to sham controls on day 4 after challenge (Extended Data Fig. 10). Ad26-S.PP vaccinated animals demonstrated minimal to no evidence of viral interstitial pneumonia, disruption of the bronchiolar epithelium or peribronchiolar aggregates of $CD3^+$ T lymphocytes and macrophages. Histiocytic and neutrophilic inflammatory infiltrates were markedly reduced in all lung lobes, and significantly reduced SARS-CoV-2 vRNA was observed in Ad26-S.dTM.PP and Ad26-S.PP vaccinated hamsters compared to sham controls ($P = 0.004$ and $P = 0.004$, respectively, two-sided Mann–Whitney tests; Fig. 4d).

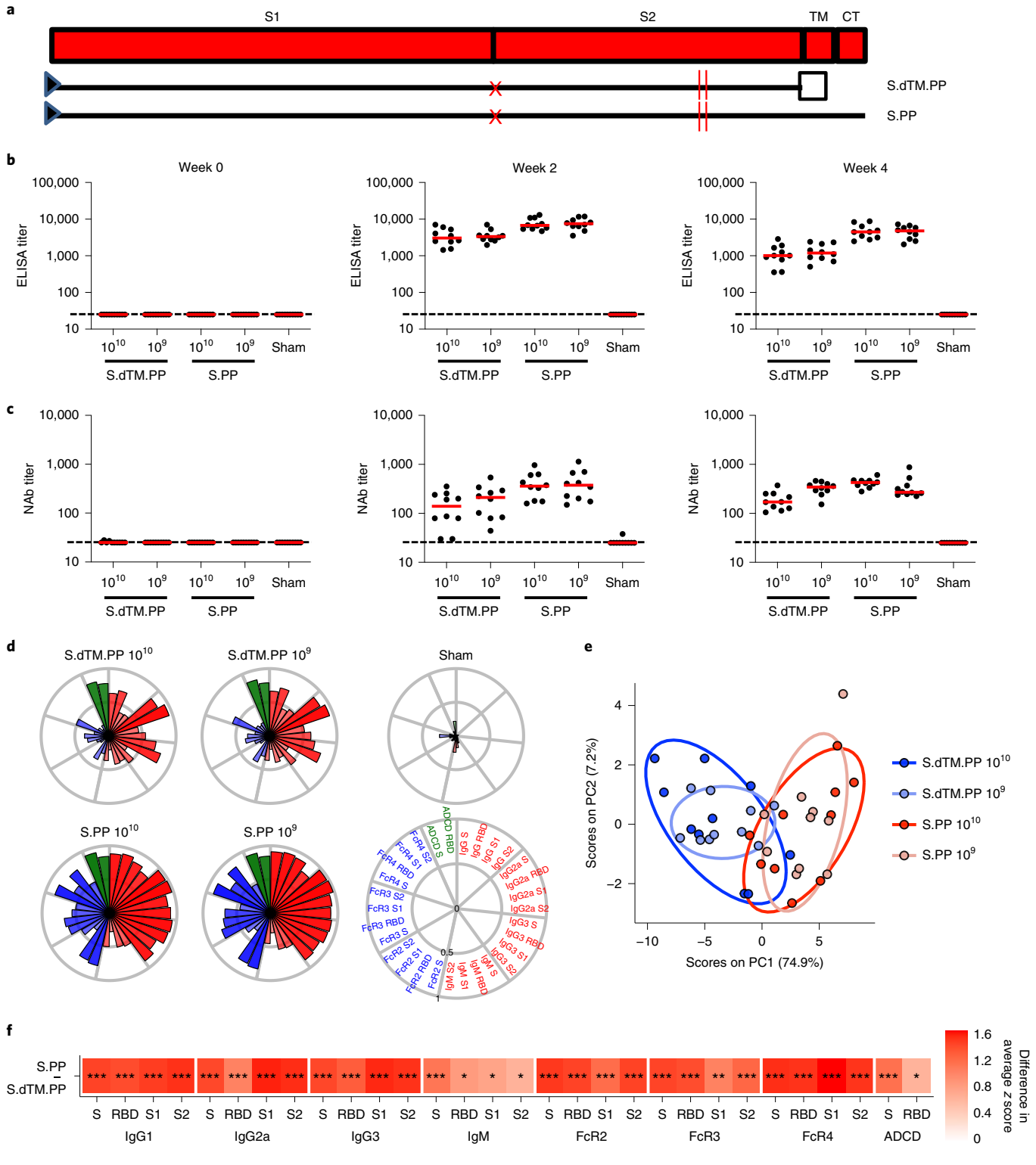
In this study, we demonstrated that a single immunization of an Ad26 vector encoding a full-length prefusion stabilized S immunogen (S.PP) protected against severe clinical disease after high-dose SARS-CoV-2 challenge in hamsters. Sham controls demonstrated marked weight loss, severe pneumonia and partial mortality. In contrast, vaccinated animals showed minimal weight loss and pneumonia and no mortality. Vaccine-elicited binding and neutralizing antibody responses correlated with protection against clinical disease as well as reduced virus replication in the upper and lower respiratory tract.

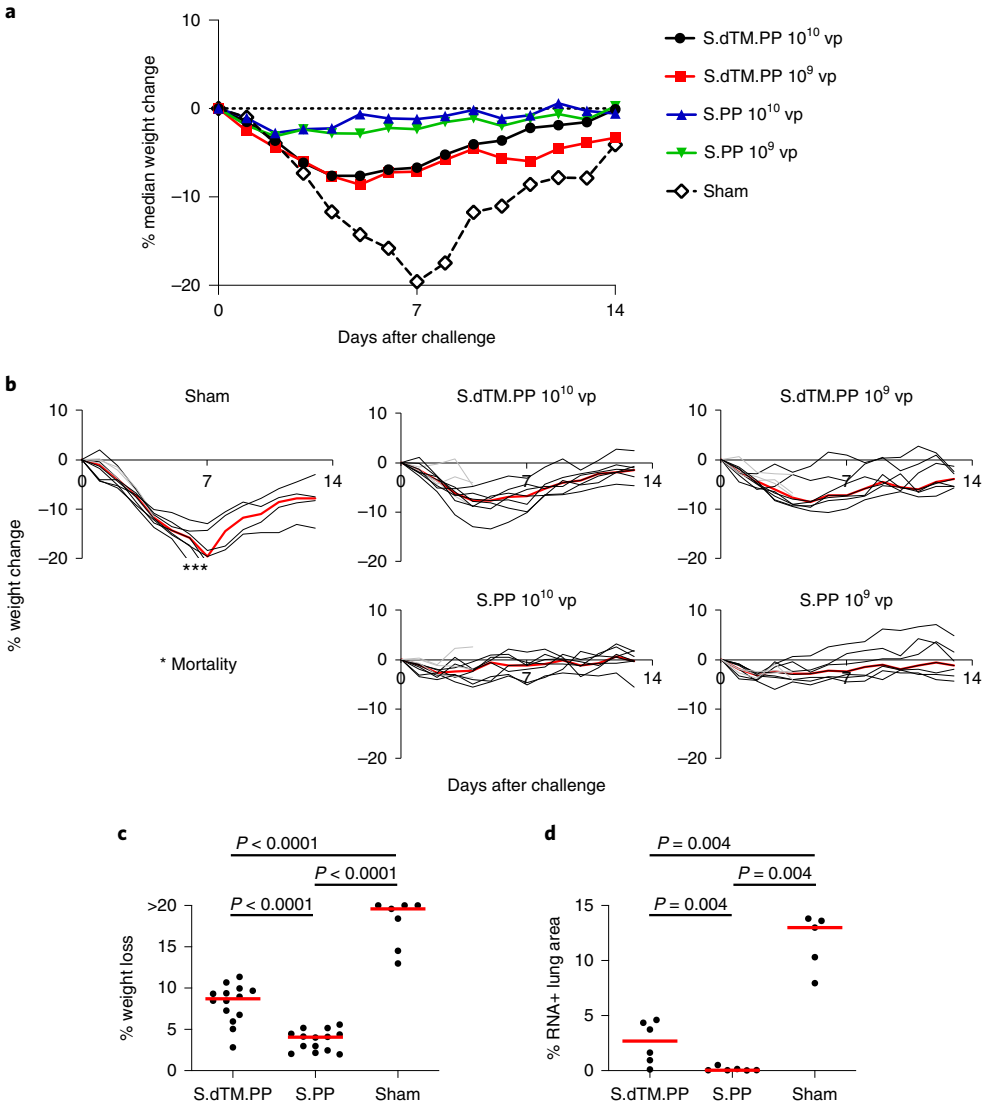
The severity of clinical disease in this model contrasts with previous studies involving SARS-CoV-2 infection in hamsters[5–7] and other species[8–10,14–18]. Hamsters are a permissive model for SARS-CoV-2 as a result of their homology to the human ACE2

Humoral immune responses in vaccinated hamsters. , SARS-CoV-2 S immunogens with 1) deletion of the transmembrane region and cytoplasmic tail reflecting the soluble ectodomain with a foldon trimerization domain (S.dTM.PP) or 2) full-length S (S.PP), both with mutation of the furin cleavage site and two proline stabilizing mutations. The red X depicts furin cleavage site mutation; red vertical lines depict proline mutations; and the open square depicts the foldon trimerization domain. S1 and S2 represent the first and second domain of the S protein; TM depicts the transmembrane region; and CT depicts the cytoplasmic domain. Hamsters were vaccinated with 10^{10} vp or 10^9 vp of Ad26-S.dTM.PP or Ad26-S.PP or sham controls ($n=10$ per group). Humoral immune responses were assessed at weeks 0, 2 and 4 by) RBD-specific binding antibody ELISA and) pseudovirus neutralization assays. Red bars reflect median responses. Dotted lines reflect assay limit of quantitation. , S- and RBD-specific IgG subclass, FcγR and ADCD responses at week 4 are shown as radar plots. The size and color intensity of the wedges indicate the median of the feature for the corresponding group (antibody subclass, red; FcγR binding, blue; ADCD, green). , PCA plot showing the multivariate antibody profiles across vaccination groups. Each dot represents an animal; the color of the dot denotes the group; and the ellipses show the distribution of the groups as 70% confidence levels assuming a multivariate normal distribution. The heat map shows the differences in the means of *z*-scored features between vaccine groups S.PP and S.dTM.PP. The two groups were compared by two-sided Mann–Whitney tests, and stars indicate the Benjamini–Hochberg-corrected *q* values (*$q<0.05$, **$q<0.01$ and ***$q<0.001$).

receptor[5], and transmission among hamsters has been reported[6]. The high challenge dose resulted in extensive clinical disease in the present study, although biologic factors that remain to be fully defined might also affect clinical disease, such as animal age, animal origin and viral challenge stock.

SARS-CoV-2 vaccine studies in nonhuman primates have, to date, demonstrated protection against infection or reduction of viral replication in the upper and lower respiratory tracts[11,12]. We have also recently reported that a single immunization of Ad26-S.PP provided complete or near-complete protection against SARS-CoV-2 challenge in rhesus macaques[13]. However, SARS-CoV-2 infection in nonhuman primates does not result in severe clinical disease or mortality[8–10]. A severe disease model would be useful to complement current nonhuman primate challenge models, because protection against viral replication does not necessarily imply protection against severe disease. Indeed, in the histopathologic analysis of





Clinical disease in hamsters after high-dose SARS-CoV-2 challenge. , Median percent weight change after challenge. , Percent weight change after challenge in individual animals. Median weight loss is depicted in red. Asterisks indicate mortality. Gray lines indicate animals with scheduled necropsies on day 4. , Maximal weight loss in the combined Ad26-S.dTM.PP ($n=14$), Ad26-S.PP ($n=14$) and sham control ($n=7$) groups, excluding the animals that were necropsied on day 4. *P* values indicate two-sided Mann–Whitney tests. *n* reflects all animals that were followed for weight loss and were not necropsied on day 4. , Quantification of percent lung area positive for anti-sense vRNA in tissue sections from Ad26-S.dTM.PP and Ad26-S.PP vaccinated hamsters as compared to control hamsters on day 4 after challenge. *P* values represent two-sided Mann–Whitney tests.

hamsters in the present study, viral loads in lung decreased from day 2 to day 7, whereas inflammatory markers continued to escalate during this time period and correlated with continued weight loss. These data suggest that progressive clinical disease in hamsters is primarily an inflammatory process, which is triggered by infection but continued to increase even when viral replication decreased.

Because COVID-19 in humans can progress to severe clinical disease, it is important to test SARS-CoV-2 vaccine candidates in preclinical models that recapitulate severe clinical disease, including fulminant pneumonia and mortality. The high-dose hamster model described here achieves many of these criteria and, therefore, might be useful to study the pathogenesis of severe disease and to test countermeasures. The primary manifestation of clinical disease in this model was severe pneumonia rather than encephalitis that has been reported in certain hACE2 transgenic mouse models[24]. Moreover, binding and neutralizing antibody responses correlated with protection.

In summary, our data demonstrate that a single immunization of Ad26-S.PP provides robust protection against severe clinical disease after high-dose SARS-CoV-2 infection in hamsters. To our knowledge, vaccine protection against severe SARS-CoV-2 pneumonia and mortality has not previously been reported. Ad26-S.PP, which is also termed Ad26.COV2.S, is currently being evaluated in clinical trials. This hamster severe disease model should prove useful for testing of SARS-CoV-2 vaccines, therapeutics and other countermeasures.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-020-1070-6.

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References

1. Li, Q. et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N. Engl. J. Med.* **382**, 1199–1207 (2020).
2. Zhu, N. et al. A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* **382**, 727–733 (2020).
3. Chen, N. et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* **395**, 507–513 (2020).
4. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497–506 (2020).
5. Chan, J. F. et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin. Infect. Dis.* https://doi.org/10.1093/cid/ciaa325 (2020).
6. Sia, S. F. et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* **583**, 834–838 (2020).
7. Imai, M. et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc. Natl Acad. Sci. USA* **117**, 16587–16595 (2020).
8. Munster, V. J. et al. et al. Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. *Nature* https://doi.org/10.1038/s41586-020-2324-7 (2020).
9. Rockx, B. et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science* **368**, 1012–1015 (2020).
10. Chandrashekar, A. et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. *Science* **369**, 812–817 (2020).
11. Yu, J. et al. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science* **369**, 806–811 (2020).
12. Gao, Q. et al. Rapid development of an inactivated vaccine candidate for SARS-CoV-2. *Science* **369**, 77–81 (2020).
13. Mercado, N. B. et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature* https://doi.org/10.1038/s41586-020-2607-z (2020).
14. Blanco-Melo, D. et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* **181**, e1039 (2020).
15. Kim, Y. I. et al. Infection and rapid transmission of SARS-CoV-2 in ferrets. *Cell Host Microbe* **27**, e702 (2020).
16. Shi, J. et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science* **368**, 1016–1020 (2020).
17. Bao, L. et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature* **583**, 830–833 (2020).
18. Sun, S. H. et al. A mouse model of SARS-CoV-2 infection and pathogenesis. *Cell Host Microbe* **28**, e124 (2020).
19. Abbink, P. et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J. Virol.* **81**, 4654–4663 (2007).
20. Wrapp, D. et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260–1263 (2020).
21. Yang, Z. Y. et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature* **428**, 561–564 (2004).
22. Chung, A. W. et al. Dissecting polyclonal vaccine-induced humoral immunity against HIV using systems serology. *Cell* **163**, 988–998 (2015).
23. Wolfel, R. et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* **581**, 465–469 (2020).
24. Netland, J., Meyerholz, D. K., Moore, S., Cassell, M. & Perlman, S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. *J. Virol.* **82**, 7264–7275 (2008).

Methods

Animals and study design. Seventy male and female Syrian golden hamsters (Envigo), 10–12 weeks old, were randomly allocated to groups. All animals were housed at Bioqual. Animals received Ad26 vectors expressing S.dTM.PP or S.PP or sham controls ($n=10$ per group). Animals received a single immunization of 10^{10} or 10^{9} vp Ad26 vectors by the intramuscular route without adjuvant at week 0. At week 4, all animals were challenged with 5.0×10^{5} $TCID_{50}$ (6×10^{8} vp, 5.5×10^{4} plaque-forming units (PFU)) or 5.0×10^{4} $TCID_{50}$ (6×10^{7} vp, 5.5×10^{3} PFU) SARS-CoV-2, which was derived with one passage from USA-WA1/2020 (NR-52281, BEI Resources)[10]. Virus was administered as 100 µl by the intranasal route (50 µl in each nare). Body weights were assessed daily. All immunologic and virologic assays were performed blinded. On day 4, a subset of animals was euthanized for tissue viral loads and pathology. All animal studies were conducted in compliance with all relevant local, state and federal regulations and were approved by the Bioqual Institutional Animal Care and Use Committee.

Ad26 vectors. Ad26 vectors were constructed with two variants of the SARS-CoV-2 S protein sequence (Wuhan/WIV04/2019; GenBank MN996528.1). Sequences were codon optimized and synthesized. Replication-incompetent, E1/E3-deleted Ad26-vectors[19] were produced in PER.C6.TetR cells using a plasmid containing the full Ad26 vector genome and a transgene expression cassette. Sham controls included Ad26-Empty vectors. Vectors were sequenced and tested for expression before use.

Histopathology and IHC. Tissues were fixed in freshly prepared 4% paraformaldehyde for 24 h, transferred to 70% ethanol, paraffin embedded within 7–10 days and block sectioned at 5 µm. Slides were baked for 30–60 min at 65 °C and then deparaffinized in xylene and rehydrated through a series of graded ethanol to distilled water. For SARS-CoV-N, Iba-1 and CD3 IHC, heat-induced epitope retrieval was performed using a pressure cooker on steam setting for 25 min in citrate buffer (Thermo Fisher Scientific, AP-9003–500), followed by treatment with 3% hydrogen peroxide. Slides were then rinsed in distilled water and protein blocked (Biocare, BE965H) for 15 min followed by rinses in 1× PBS. Primary rabbit anti-SARS-CoV-nucleoprotein antibody (Novus, NB100–56576, at 1:500 or 1:1,000), rabbit anti-Iba-1 antibody (Wako, 019–19741, at 1:500) or rabbit anti-CD3 (Dako, A0452, at 1:300) was applied for 30 min, followed by rabbit Mach-2 HRP-Polymer (Biocare, RHRP520L) for 30 min and then counterstained with hematoxylin followed by bluing using 0.25% ammonia water. Labeling for SARS-CoV-N, Iba-1 and CD3 was performed on a Biogenex i6000 Autostainer (v3.02). In some cases, CD3, Iba-1 and ACE-2 staining was performed with CD3 at 1:400 (Thermo Fisher Scientific, cat. no. RM-9107-S, clone SP7), Iba-1 at 1:500 (Biocare, cat. no. CP290A, polyclonal) or ACE-2 (Abcam, ab108252), all of which were detected by using Rabbit Polink-1 HRP (GBI Labs, cat. no. D13–110). Neutrophil (MPO) and type 1 IFN response (Mx1) was performed with MPO (Dako, cat. no. A0398, polyclonal) at 1:1,000 detection using Rabbit Polink-1 HRP and Mx1 (EMD Millipore, cat. no. MABF938, clone M143/CL143) at 1:1,000 detection using Mouse Polink-2 HRP (GBI Labs, cat. no. D37–110). Staining for CD3, Iba-1, MPO and Mx1 IHC was performed as previously described using a Biocare intelliPATH autostainer, with all antibodies being incubated for 1 h at room temperature. Tissue pathology was assessed independently by two veterinary pathologists (A.J.M. and C.P.M.).

RNAscope in situ hybridization. RNAscope in situ hybridization was performed as previously described[10] using SARS-CoV2 anti-sense specific probe v-nCoV2019-S (ACD, cat. no. 848561) targeting the positive-sense viral RNA and SARS-CoV2 sense specific probe v-nCoV2019-orf1ab-sense (ACD, cat. no. 859151) targeting the negative-sense genomic vRNA. In brief, after slides were deparaffinized in xylene and rehydrated through a series of graded ethanol to distilled water, retrieval was performed for 30 min in ACD P2 retrieval buffer (ACD, cat. no. 322000) at 95–98 °C, followed by treatment with protease III (ACD, cat. no. 322337) diluted 1:10 in PBS for 20 min at 40 °C. Slides were then incubated with 3% H_2O_2 in PBS for 10 min at room temperature. Before hybridization, probe stocks were centrifuged at 13,000 r.p.m. using a microcentrifuge for 10 min and then diluted 1:2 in probe diluent (ACD, cat. no. 300041) to reduce probe aggregation tissue artifacts. Slides were developed using the RNAscope 2.5 HD Detection Reagents-RED (ACD, cat. no. 322360).

Quantitative image analysis. Quantitative image analysis was performed using HALO software (v2.3.2089.27 or v3.0.311.405; Indica Labs) on at least one lung lobe cross-section from each animal. In cases where more than one cross-section was available, each lung lobe was quantified as an individual data point. For SARS-CoV-N, the Multiplex IHC v2.3.4 algorithm was used with an exclusion screen for acid hematin to determine the percentage of SAR-N protein positive cells as a proportion of the total number of cells. For Iba-1, the Multiplex IHC v2.3.4 algorithm was used for quantitation. For SARS-CoV-2 RNAscope ISH and Mx1 quantification, the Area Quantification v2.1.3 module was used to determine the percentage of total SARS-CoV-2 anti-sense or sense probe or Mx1 protein as a proportion of the total tissue area. For MPO (neutrophil) and $CD3^{+}$ cell quantification, slides were annotated to exclude blood vessels (>5 mm^2), bronchi, bronchioles, cartilage and connective tissue; subsequently, the Cytonuclear v1.6 module was used to detect MPO^{+} or $CD3^{+}$ cells and frequency was calculated as a proportion of total alveolar tissue (polymorphonuclear leukocytes per mm^2), determined by running the Area Quantification v2.1.3 module. In all instances, manual inspection of all images was performed on each sample to ensure that the annotations were accurate.

Subgenomic mRNA assay. SARS-CoV-2 E gene subgenomic mRNA (sgmRNA) was assessed by RT–PCR using primers and probes as previously described[10,11,23]. Briefly, total RNA was extracted from tissue homogenates from several anatomical sites using a QIAcube HT (Qiagen) and RNeasy 96 QIAcube HT Kit (Qiagen). A standard curve was generated using the SARS-CoV-2 E gene sgmRNA by cloning into a pcDNA3.1 expression plasmid; this insert was transcribed using an AmpliCap-Max T7 High Yield Message Maker Kit (Cellscript). Before RT–PCR, samples collected from challenged animals or standards were reverse transcribed using Superscript III VILO (Invitrogen) according to the manufacturer's instructions. A Taqman custom gene expression assay (Thermo Fisher Scientific) was designed using the sequences targeting the E gene sgmRNA. Reactions were carried out on QuantStudio 6 and 7 Flex Real-Time PCR Systems (Applied Biosystems) according to the manufacturer's specifications. Standard curves were used to calculate sgmRNA copies per gram tissue; the quantitative assay sensitivity was 100 copies.

ELISA. RBD-specific binding antibodies were assessed by ELISA essentially as described[10,11]. Briefly, 96-well plates were coated with 1 µg ml^{-1} of SARS-CoV-2 RBD protein (Aaron Schmidt, Massachusetts Consortium on Pathogen Readiness) or 1 µg ml^{-1} of SARS-CoV-2 S protein (Sino Biological) in 1× Dulbecco's phosphate-buffered saline (DPBS) and incubated at 4 °C overnight. After incubation, plates were washed once with wash buffer (0.05% Tween-20 in 1× DPBS) and blocked with 350 µl of casein block per well for 2–3 h at room temperature. After incubation, the block solution was discarded and plates were blotted dry. Three-fold serial dilutions of heat-inactivated serum in casein block were added to wells, and plates were incubated for 1 h at room temperature. Plates were washed three times and then subsequently incubated for 1 h with 0.1 µg ml^{-1} of anti-hamster IgG HRP (SouthernBiotech) in casein block at room temperature in the dark. Plates were washed three times, and then 100 µl of SeraCare KPL TMB SureBlue Start solution was added to each well; plate development was halted by the addition of 100 µl of SeraCare KPL TMB Stop solution per well. The absorbance at 450 nm was recorded using a VersaMax or Omega microplate reader. ELISA endpoint titers were defined as the highest reciprocal serum dilution that yielded an absorbance two-fold above background.

Pseudovirus neutralization assay. The SARS-CoV-2 pseudoviruses expressing a luciferase reporter gene were generated in an approach similar to as described previously[10,11,21]. Briefly, the packaging construct psPAX2 (AIDS Resource and Reagent Program), luciferase reporter plasmid pLenti-CMV Puro-Luc (Addgene) and S protein expressing pcDNA3.1-SARS CoV-2 SΔCT were co-transfected into HEK293T cells by lipofectamine 2000 (Thermo Fisher Scientific). The supernatants containing the pseudotype viruses were collected 48 h after transfection; pseudotype viruses were purified by filtration with a 0.45-µm filter. To determine the neutralization activity of the antisera from vaccinated animals, HEK293T-hACE2 cells were seeded in 96-well tissue culture plates at a density of 1.75×10^{4} cells per well overnight. Three-fold serial dilutions of heat-inactivated serum samples were prepared and mixed with 50 µl of pseudovirus. The mixture was incubated at 37 °C for 1 h before adding to HEK293T-hACE2 cells. Forty-eight hours after infection, cells were lysed in Steady-Glo Luciferase Assay (Promega) according to the manufacturer's instructions. SARS-CoV-2 neutralization titers were defined as the sample dilution at which a 50% reduction in relative light units was observed relative to the average of the virus control wells.

Luminex. To detect relative quantity of antigen-specific antibody titers, a customized Luminex assay was performed as previously described[25]. Hereby, fluorescently labeled microspheres (Luminex) were coupled with SARS-CoV-2 antigens including S protein (Eric Fischer, Dana Farber Cancer Institute), S1 and S2 (Sino Biological), as well as RBD (Aaron Schmidt, Ragon Institute) via covalent N-hydroxysuccinimide (NHS)–ester linkages via EDC (Thermo Fisher Scientific) and Sulfo-NHS (Thermo Fisher Scientific). Then, 1.2×10^{3} beads per region and antigen were added to a 384-well plate (Greiner) and incubated with diluted serum (1:90 for IgG2a, IgG3, IgM; 1:500 for total IgG and Fc-receptor binding assays) for 16 h shaking at 900 r.p.m. at 4 °C. After formation of immune complexes, microspheres were washed three times in 0.1% bovine serum albumin and 0.05% Tween-20 (Luminex assay buffer) using an automated plate washer (Tecan). PE-labeled goat anti-mouse IgG, IgG2a, IgG3 and IgM detection antibodies (SouthernBiotech) were diluted in Luminex assay buffer to 0.65 µg ml^{-1} and incubated with beads for 1 h at room temperature while shaking at 900 r.p.m. Similarly, for the Fc-receptor binding profiles, recombinant mouse FcγR2, FcγR3 and FcγR4 (Duke Protein Production Facility) were biotinylated (Thermo Fisher Scientific) and conjugated to Streptavidin-PE for 10 min before addition to samples (SouthernBiotech). These mouse antibodies and proteins are cross-reactive to hamster. The coated beads were then washed and read on a flow cytometer, iQue

(IntelliCyt), with a robot arm attached (PAA). Events were gated on each bead region; median fluorescence of PE of bead-positive events was reported. Samples were run in duplicate per each secondary detection agent.

ADCD. ADCD assays were performed as previously described[26]. Briefly, SARS-CoV-2 S and RBD were biotinylated (Thermo Fisher Scientific) and coupled to 1 µm red fluorescent neutravidin beads (Thermo Fisher Scientific) for 2 h at 37 °C, and excess antigen was washed away afterwards. For the formation of immune complexes, 1.82×10^8 antigen-coated beads were added to each well of a 96-well round bottom plate and incubated with 1:10 diluted samples at 37 °C for 2 h. Lyophilized guinea pig complement was reconstituted according to the manufacturer's instructions (Cedarlane) with water, and 4 µl per well was added in gelatin veronal buffer containing Mg^{2+} and Ca^{2+} (GVB^{++}, Boston BioProducts) to the immune complexes for 20 min at 37 °C. Immune complexes were washed with 15 mM ethylenediaminetetraacetic acid in PBS, and fluorescein-conjugated goat IgG fraction to guinea pig complement C3 (MP Biomedicals) was added. After staining, samples were fixed with 4% paraformaldehyde, and sample acquisition was performed via flow cytometry (IntelliCyt, iQue Screener Plus) using a robot arm (PAA). All events were gated on single cells and bead-positive events; the median of C3-positive events is reported. All samples were run in duplicate on separate days.

Statistical analysis. Analysis of immunologic, virologic and body weight data was performed using GraphPad Prism 8.4.2 (GraphPad Software). Comparison of data between groups was performed using two-sided Mann–Whitney tests. Mortality was assessed by two-sided Fisher's exact tests. Correlations were assessed by two-sided Spearman's rank correlation tests. *P* values of less than 0.05 were considered significant. All systems serology data were $\log_{10}$ transformed. For the radar plots, each antibody feature was normalized such that its minimal value is 0 and the maximal value is 1 across groups before using the median within a group. A PCA was constructed using the R version 3.6.1 package 'ropls' to compare multivariate profiles. For the visualization in the heat map, the differences in the means of the S.dTM.PP and S.PP groups of *z*-scored features were shown. To indicate significances in the heat maps, a Benjamini–Hochberg correction was used to correct for multiple comparisons within a row.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data are available in the manuscript or the supplementary material. Correspondence and requests for materials should be addressed to D.H.B. (dbarouch@bidmc.harvard.edu).

References

25. Brown, E. P. et al. High-throughput, multiplexed IgG subclassing of antigen-specific antibodies from clinical samples. *J. Immunol. Methods* **386**, 117–123 (2012).

26. Fischinger, S. et al. A high-throughput, bead-based, antigen-specific assay to assess the ability of antibodies to induce complement activation. *J. Immunol. Methods* **473**, 112630 (2019).

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Author contributions

L.H.T., H.A., M.G.L., F.W., R.Z. and D.H.B. designed the study and reviewed all data. F.W., J.C., H.S. and R.Z. designed the vaccines. A.J.M., C.N.C., S.B., C.E.S., M.N., K.B.-S., C.P.-M., L.M.W., S.D. and J.D.E. performed the pathology. L.H.T., K.M., N.B.M., J.Y., E.A.B., G.D., M.S.G., C.J.D., Z.L., S.H.M., F.N. and R.N. performed the immunologic and virologic assays. C.L., C.A., S.F., J.S.B., D.A.L. and G.A. performed the systems serology. L.P., M.P., V.A., D.B., K.T., H.A. and M.G.L. led the clinical care of the animals. J.F., B.M.H., T.M.C., Y.C., B.C. and A.G.S. provided purified proteins. D.H.B. wrote the paper with all co-authors.

Competing interests

D.H.B., F.W., J.C., H.S. and R.Z. are co-inventors on related vaccine patents. F.W., J.C., H.S. and R.Z. are employees of Janssen Vaccines & Prevention BV and hold stock in Johnson & Johnson.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-020-1070-6.

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Correspondence and requests for materials should be addressed to D.H.B.

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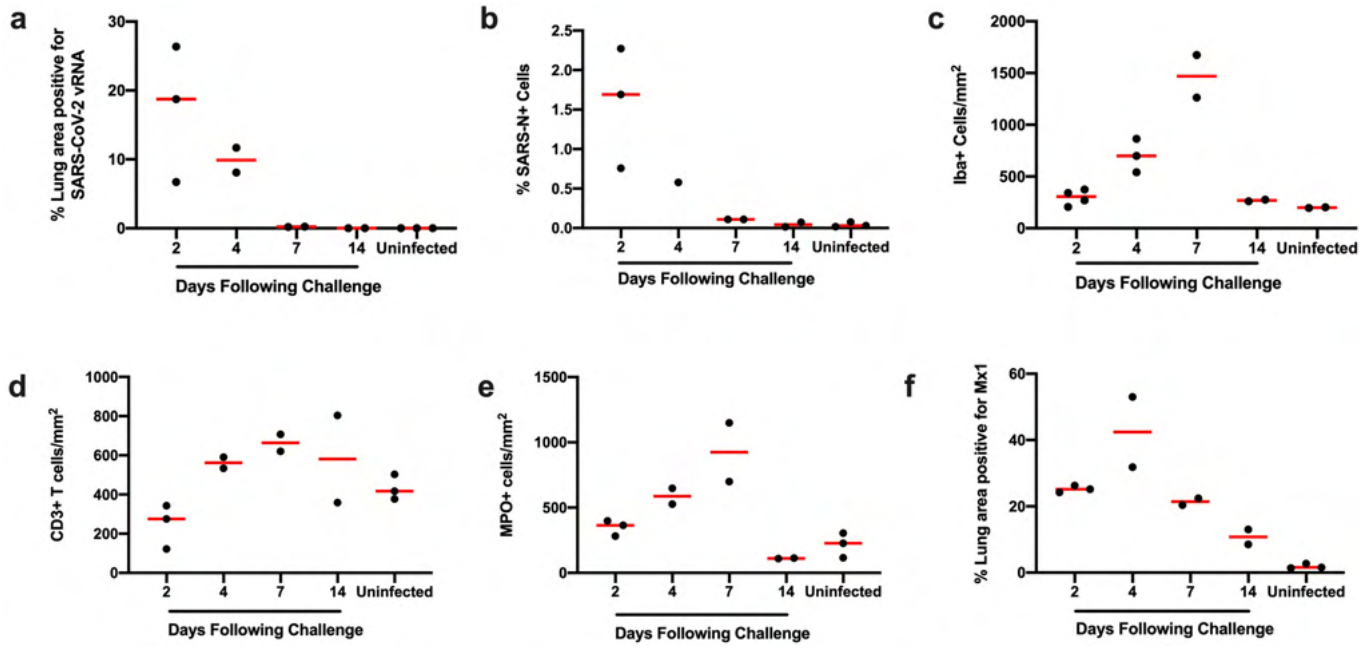
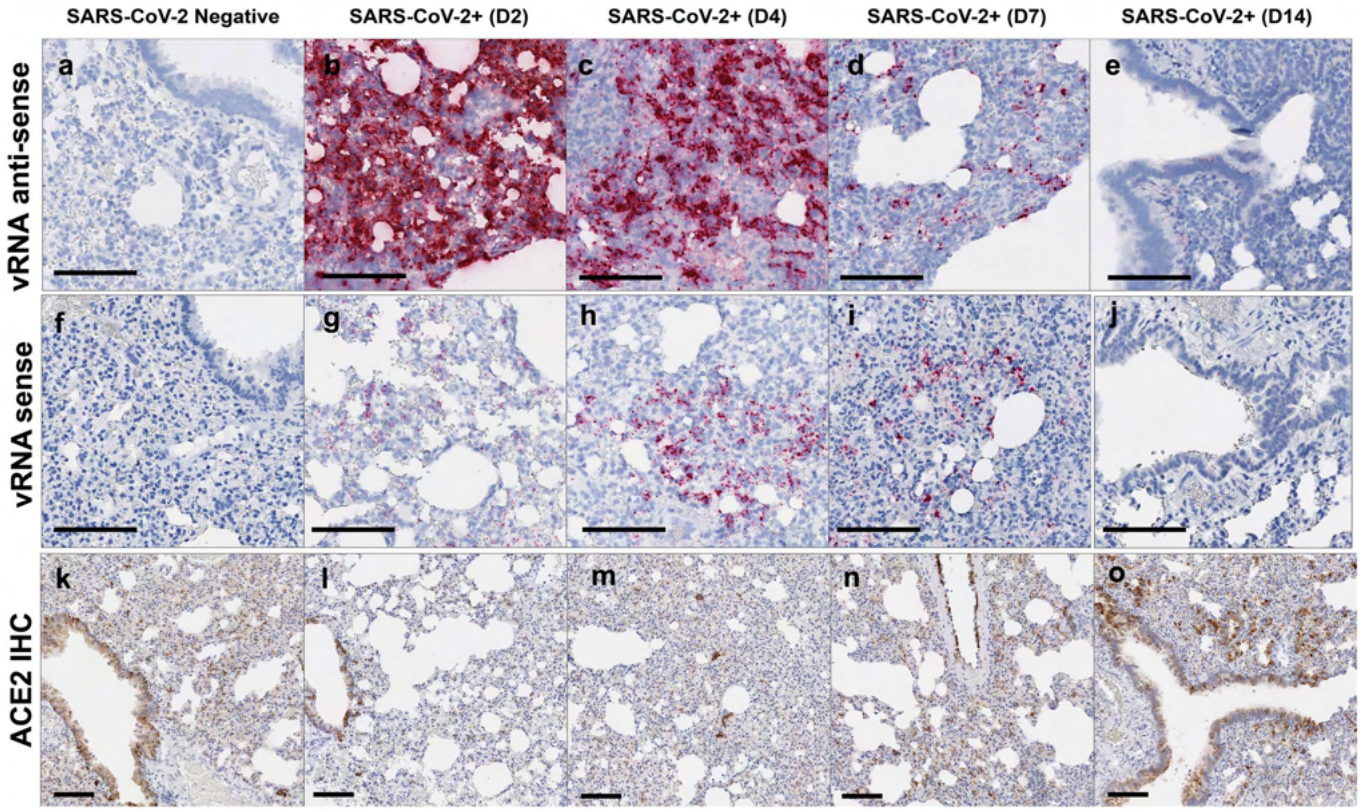
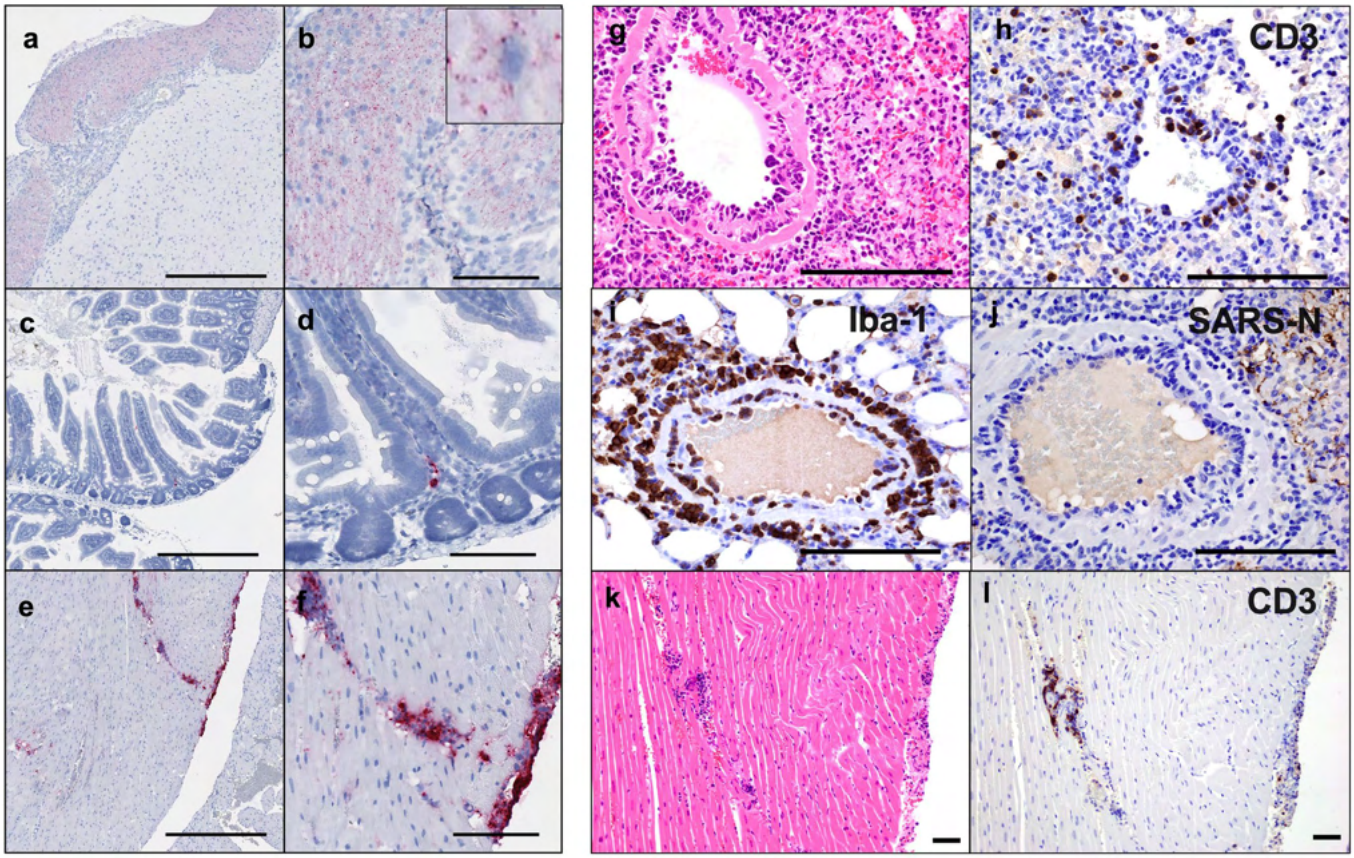


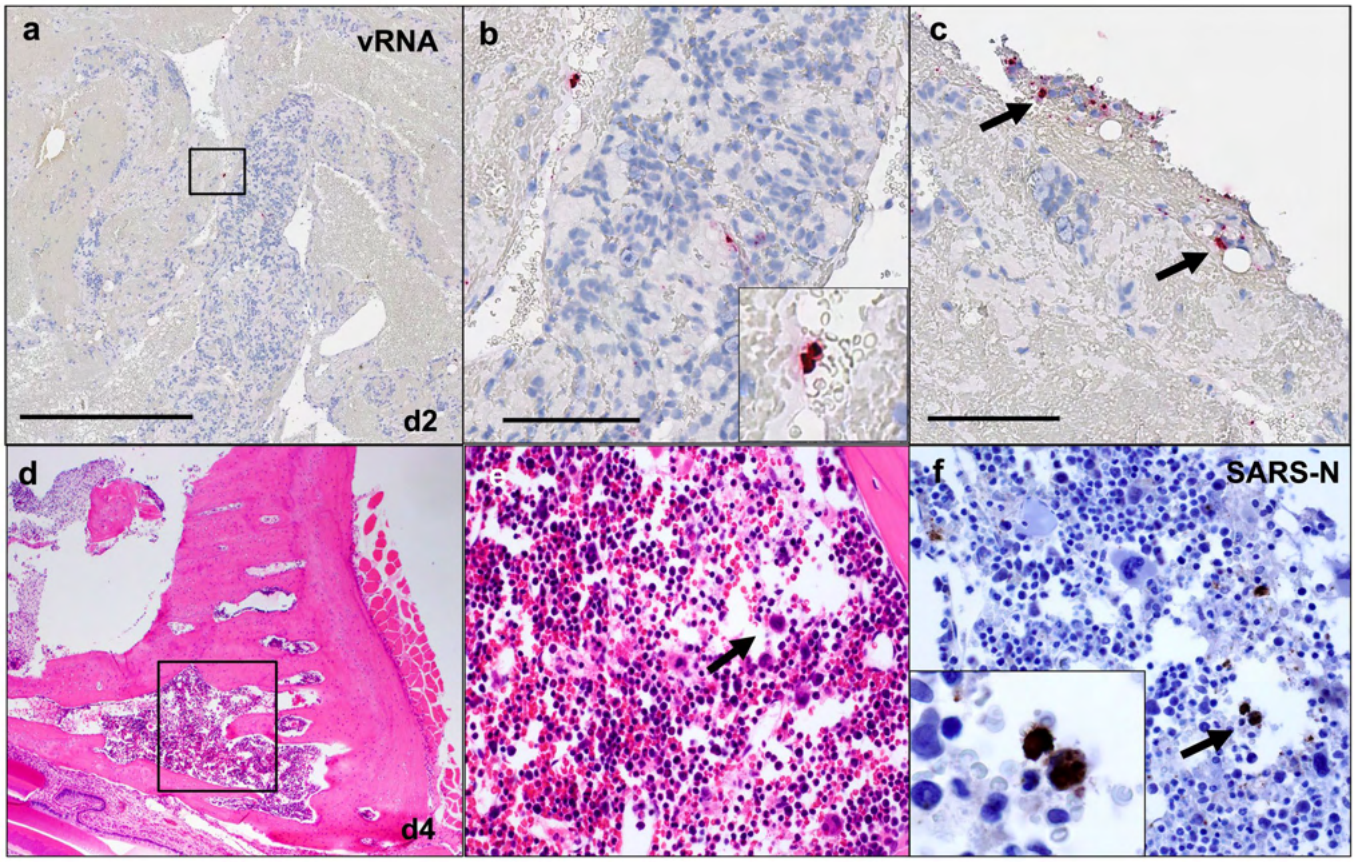
E□□□□□□ D□□ □□□□□Longitudinal quantitative image analysis of viral replication and associated inflammation in lungs. □, Percent lung area positive for anti-sense SARS-CoV-2 viral RNA (vRNA) by RNAscope ISH. □, Percentage of total cells positive for SARS-CoV-N protein (nuclear or cytoplasmic) by IHC. □, Iba-1 positive cells per unit area by IHC. □, CD3 positive cells per unit area. □, MPO positive cells per unit area. □ Percentage of MX1 positive lung tissue as a proportion of total lung area. ISH, in situ hybridization; IHC, immunohistochemistry; SARS-N, SARS-CoV nucleocapsid; MPO, myeloperoxidase; MX1, myxovirus protein 1 (a type 1 interferon inducible gene). Each dot represents one animal.



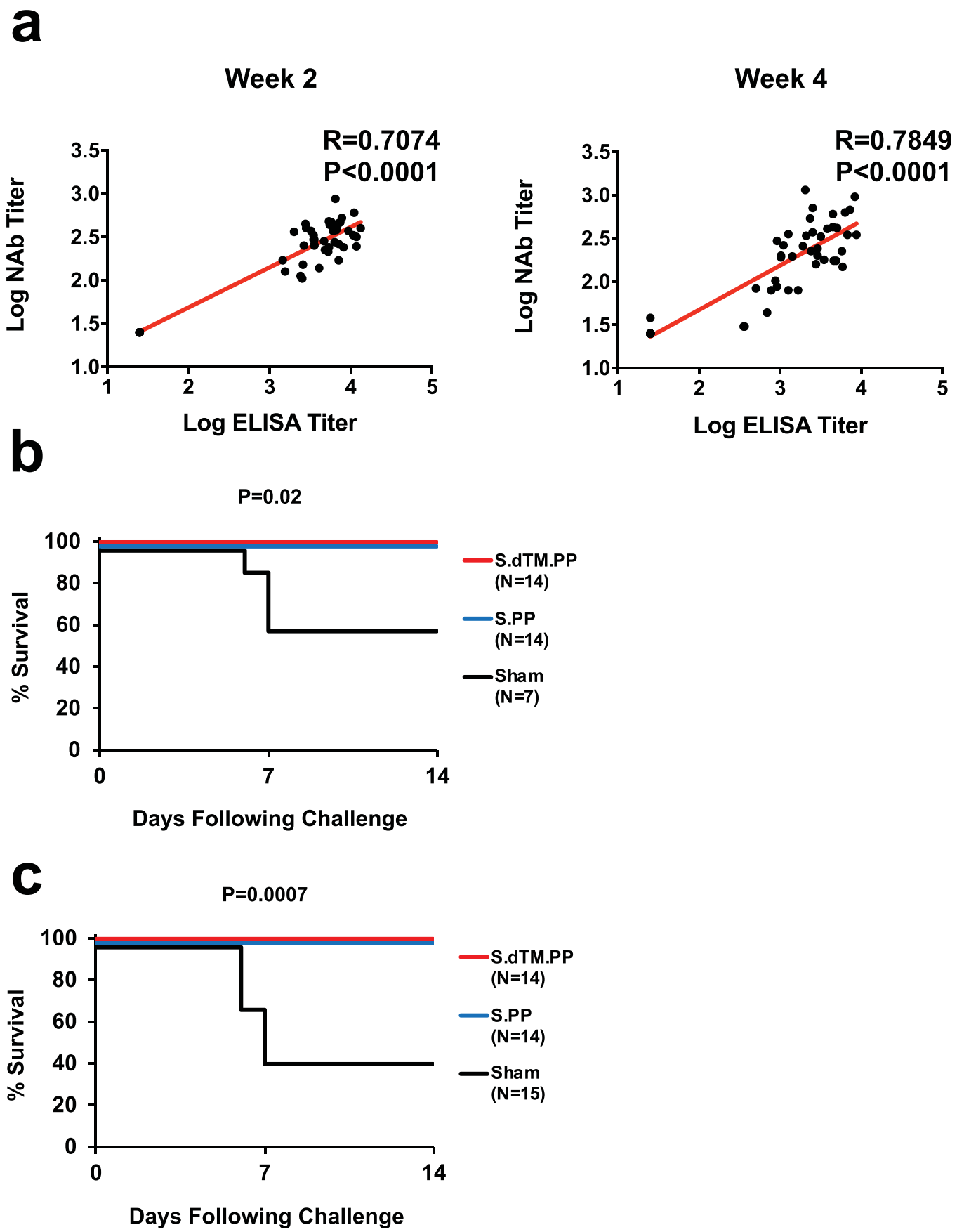
E□□□□□□□ D□□□ □□□□□ □Lung viral dynamics and ACE2 receptor expression patterns. Hamsters were necropsied before (SARS-CoV-2 Negative) or after high-dose SARS-CoV-2 challenge on day 2 (D2), day 4 (D4), day 7 (D7), and day 14 (D14) following challenge. Serial sections of lung tissue were stained for vRNA anti-sense RNAscope **a-e**, for vRNA sense RNAscope **f-j**, and ACE2 IHC **k-o**. Anti-sense RNAscope used a sense probe; sense RNAscope used an anti-sense probe. IHC, immunohistochemistry. Representative sections are shown. Experiments were repeated at least 3 times with similar results. Scale bars = 100 μm.



E□□□□□□□ D□□□ □□□□□ □Extrapulmonary pathology. □, Anti-sense SARS-CoV-2 viral RNA (vRNA) in brainstem on day 2 following challenge. □, Higher magnification showing cytoplasmic vRNA staining in neurons in the absence of inflammation and pathology. □, Anti-sense SARS-CoV-2 vRNA staining in the lamina propria of small intestinal villus on day 2 following challenge. □, Higher magnification showing cytoplasmic and nuclear vRNA staining in an individual mononuclear cell in the absence of inflammation and tissue pathology. □, Anti-sense SARS-CoV-2 vRNA staining within the myocardium and along the epicardial surface of the heart on day 4 following challenge. □ Higher magnification showing staining of inflammatory mononuclear cell infiltrates consistent with focal myocarditis. □, Pulmonary vessel showing endothelialitis day 4 (d4) following challenge. □, Pulmonary vessel showing CD3+ T lymphocyte staining by IHC adhered to endothelium and within vessel wall, d4 following challenge. □ Pulmonary vessel showing Iba-1+ staining by IHC of macrophages along endothelium and perivascularly, d4. □ Pulmonary vessel showing minimal vascular staining for SARS-CoV-N by IHC, d4. □, Heart from (□, □) showing focal lymphocytic myocarditis as confirmed by CD3+ T lymphocyte staining □ of cells by IHC, d4. Representative sections are shown. Experiments were repeated at least 3 times with similar results. Scale bars = 500 μm (**a, c, e**); 100 μm (**b, d, f, g-l**).



E□□□□□□□ D□□ □□□□ □SARS-CoV-2 in blood mononuclear cells and bone marrow. a-c, SARS-CoV-2 anti-sense vRNA staining within mononuclear cells within lung thrombus on day 2 following challenge. □, Bone marrow from the nasal turbinate 4 days following challenge showing □, hematopoetic cells (H&E) that show □ positive staining for SARS-CoV-N IHC. vRNA, viral RNA; H&E, hematoxylin and eosin; IHC, immunohistochemistry. Representative sections are shown. Experiments were repeated at least 3 times with similar results. Scale bars = 500 μm (□); 200 μm (□); 100 μm (**b, c, e, f**).


a
Week 2
R=0.7074
P<0.0001
Log NAb Titer
Log ELISA Titer
Week 4
R=0.7849
P<0.0001
Log NAb Titer
Log ELISA Titer
b
P=0.02
% Survival
Days Following Challenge
S.dTM.PP (N=14)
S.PP (N=14)
Sham (N=7)
c
P=0.0007
% Survival
Days Following Challenge
S.dTM.PP (N=14)
S.PP (N=14)
Sham (N=15)

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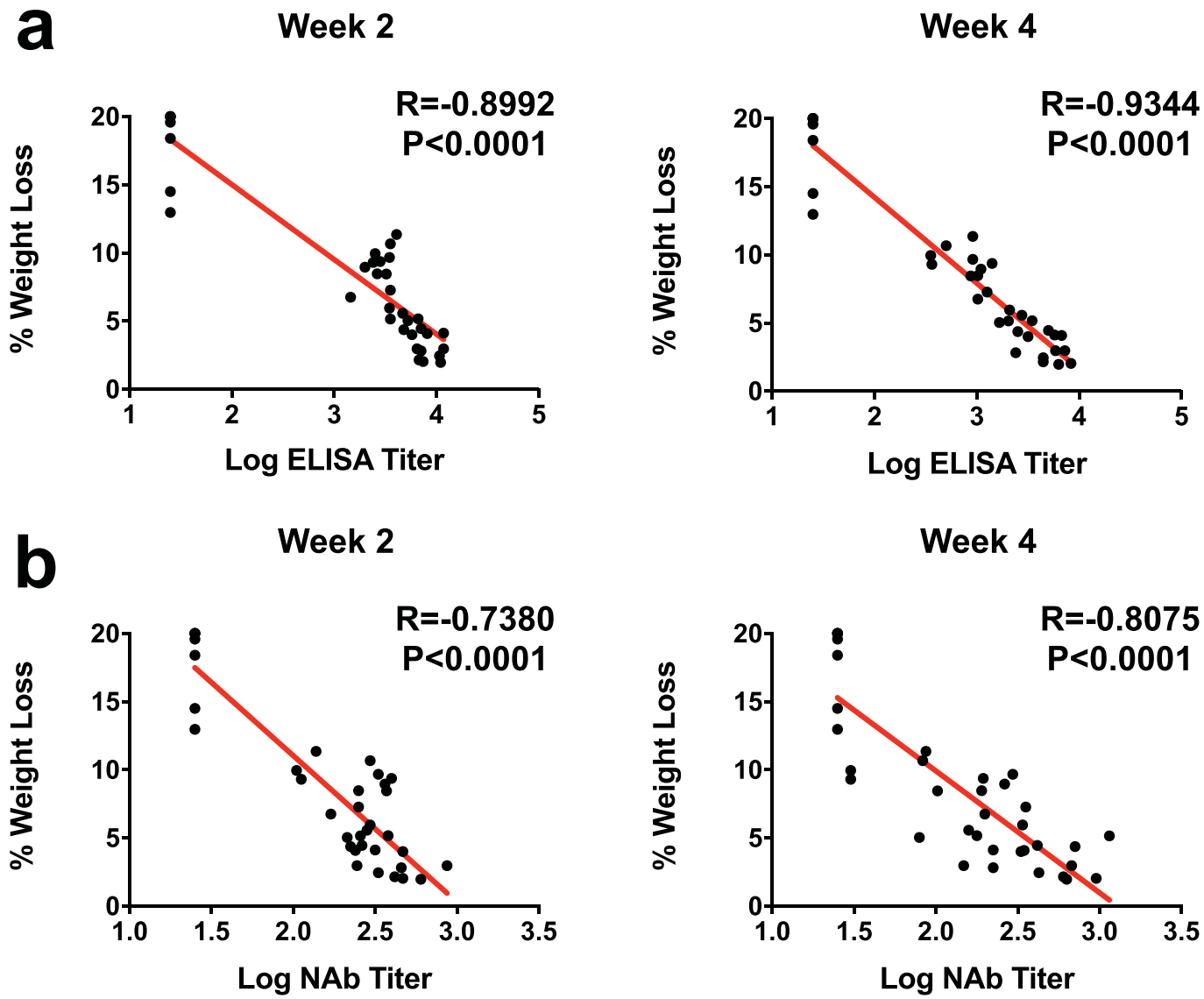
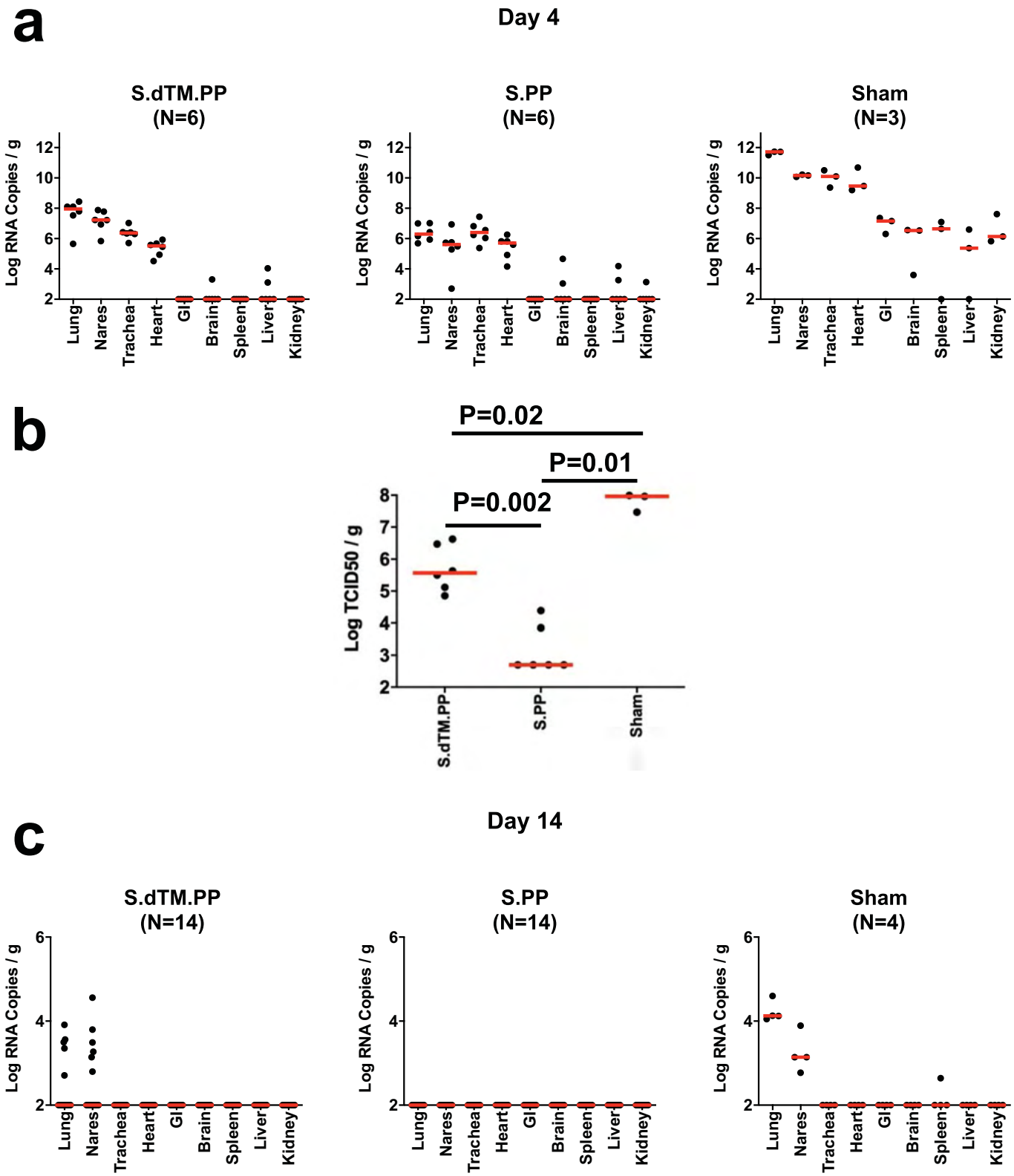
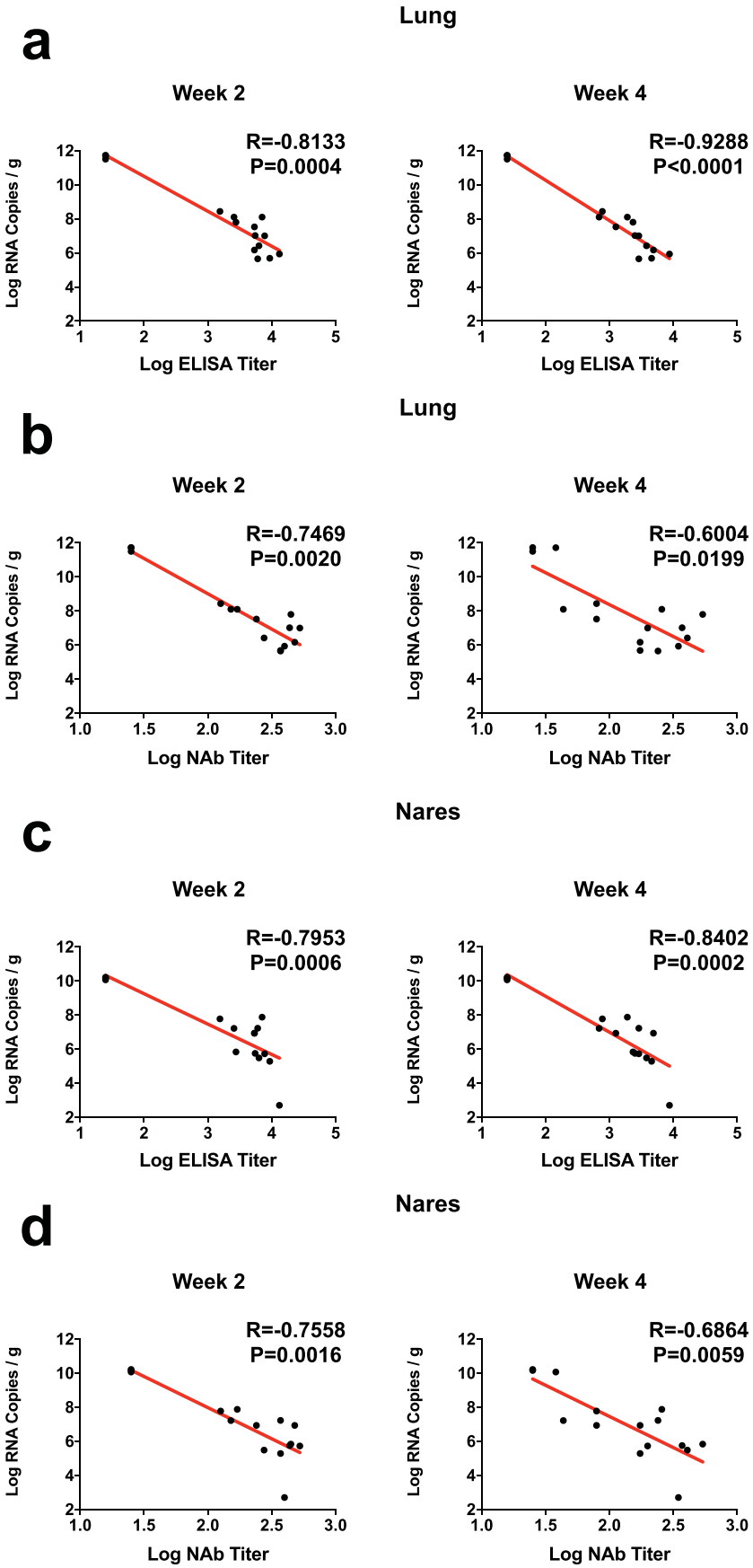
E□□□□□□ D□□ □□□□ □Correlation of antibody titers and survival curves. □, Correlations of binding ELISA titers and pseudovirus NAb titers at week 2 and week 4. Red lines reflect the best linear fit relationship between these variables. P and R values reflect two-sided Spearman rank-correlation tests. □, Survival curve for the vaccine study. P values indicate two-sided Fisher's exact tests. N denotes number of animals in each group. □, Combined analysis of the two hamster studies involving all animals that received the $5x10^5$ $TCID_{50}$ challenge dose and were followed longitudinally. P values indicate two-sided Fisher's exact tests. N denotes number of animals in each group.



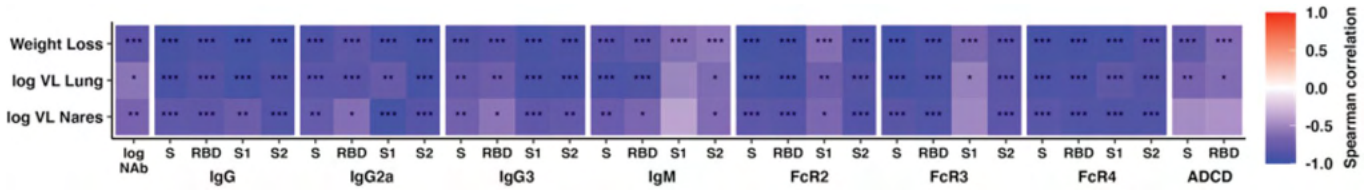
E□□□□□□ D□□ □□□□ □Antibody correlates of clinical protection. Correlations of □, binding ELISA titers and □, pseudovirus NAb titers at week 2 and week 4 with maximum percent weight loss following challenge. Red lines reflect the best linear fit relationship between these variables. P and R values reflect two-sided Spearman rank-correlation tests.

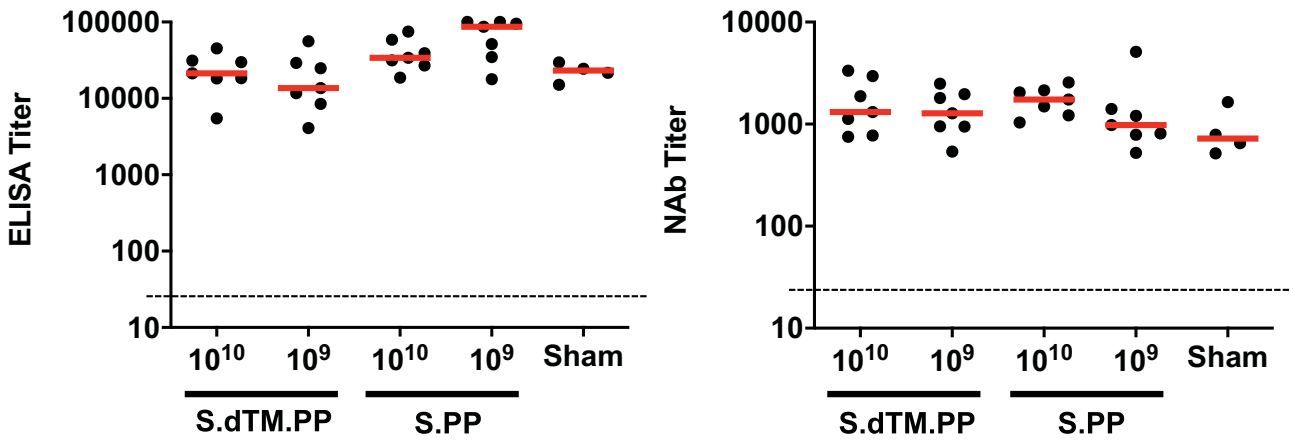


E□□□□□□ D□□ □□□□ □Tissue viral loads on day 4 and day 14. Tissue viral loads as measured by □, log_{10} subgenomic RNA copies per gram tissue (limit of quantification 100 copies/g) and □, log_{10} infectious virus TCID50 titers per gram tissue (limit of quantification 100 TCID50/g) on day 4 (N=6 reflects both dose groups for each vaccine) and □, log_{10} subgenomic RNA copies per gram tissue on day 14 (N=14 reflects both dose groups for each vaccine) following challenge. Red lines reflect median values. Each dot represents one animal.

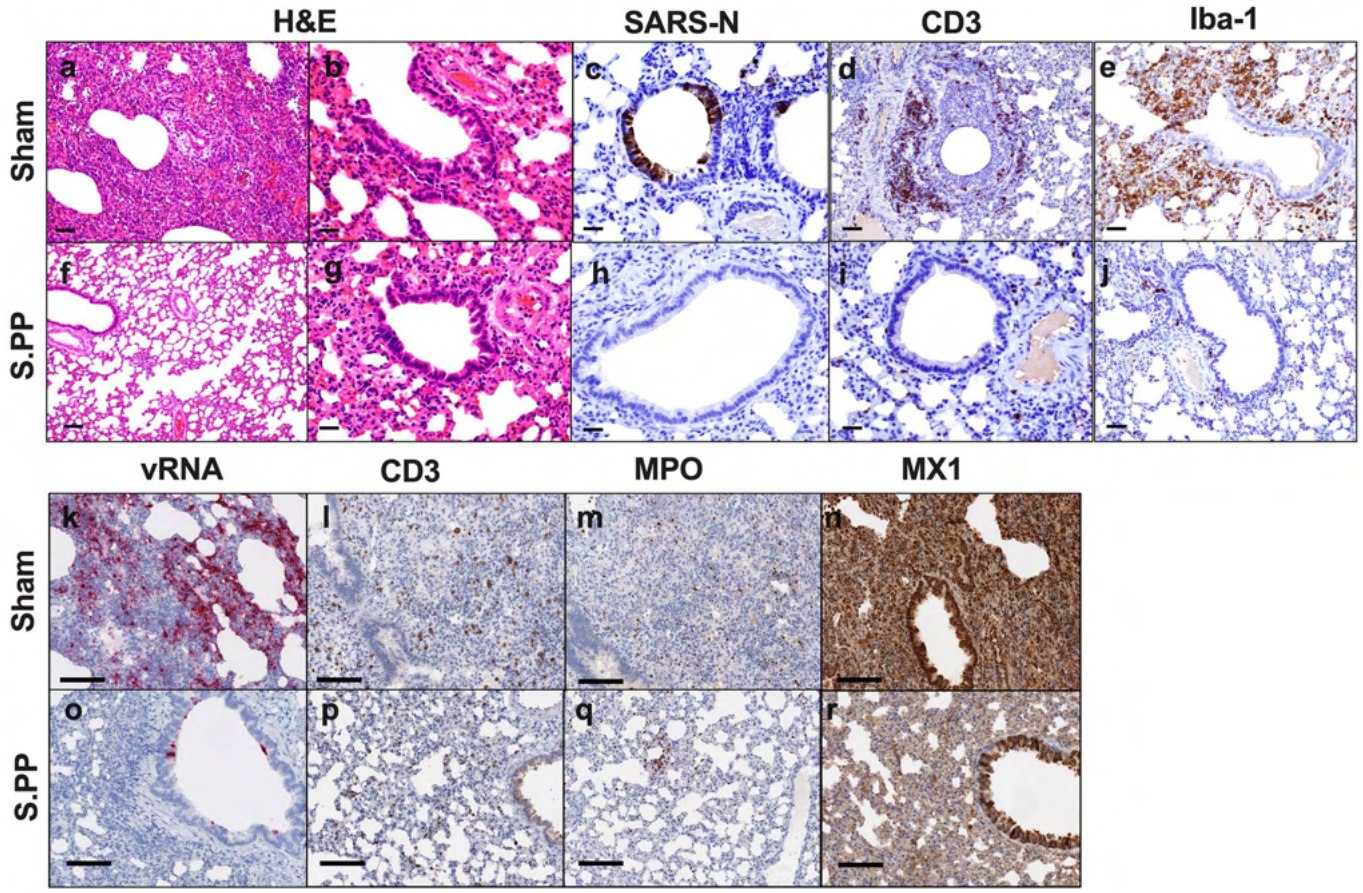


E□□□□□□ D□□ □□□□ □Antibody correlates of protection. Correlations of **a, c**, binding ELISA titers and **b, d**, pseudovirus NAb titers at week 2 and week 4 with $\log_{10}$ RNA copies per gram (**a, b**) lung and (**c, d**) nasal turbinate tissue in the animals that were necropsied on day 4. Red lines reflect the best linear fit relationship between these variables. P and R values reflect two-sided Spearman rank-correlation tests.





E□□□□□□□ D□□□ □□□□ □Antibody correlates of protection and anamnestic responses. □, The heatmaps show the Spearman rank correlation between antibody features and weight loss (N=35), lung viral loads (N=12), and nasal turbinate viral loads (N=12). N reflects all animals that were followed for weight loss or that were necropsied for lung or nasal turbinate viral loads. Significant correlations are indicated by stars after multiple testing correction using the Benjamini-Hochberg procedure (*q < 0.05, ** q < 0.01, *** q < 0.001). □, ELISA and NAb responses in surviving hamsters on day 14 following SARS-CoV-2 challenge.



E□□□□□□ D□□ □□□□□ □Ad26 vaccination protects against SARS-CoV-2 pathology. Histopathological H&E evaluation of **a–e, k–n**, sham controls and **f–j, o–r**, Ad26-S.PP vaccinated hamsters shows in sham controls (□) severe consolidation of lung parenchyma and infiltrates of inflammatory cells, (□) bronchiolar epithelial syncytia and necrosis, (□) SARS-CoV-N positive (IHC) bronchiolar epithelial cells, (□) peribronchiolar CD3+ T lymphocyte infiltrates, and (□) peribronchiolar macrophage infiltrates (Iba-1; IHC), and (**f-j**) minimal to no corresponding pathology in Ad26-S.PP vaccinated animals. SARS-CoV-2 anti-sense RNAscope ISH in (□), interstitial CD3+ T lymphocytes □ MPO staining by IHC, and MX1 staining by IHC □) in sham controls compared to similar regions in Ad26-S.PP vaccinated animals **o-r**) on day 4 following challenge. Representative sections are shown. Experiments were repeated at least 3 times with similar results. Scale bars = 20 μm (**b, c, g–i**); 50 μm (**a, d, e, j**); 100 μm (**f, k–r**).

nature research

Corresponding author(s): Dan Barouch

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☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect data.
Data analysis	Analysis of virologic and immunologic data was performed using R 3.6.1 and GraphPad Prism 8.4.2 (GraphPad Software).

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All data are available in the manuscript or the supplementary material. Correspondence and requests for materials should be addressed to D.H.B. (dbarouch@bidmc.harvard.edu).

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size includes N=70 animals (N=10 animals/group given our experience with these vaccines in Mercado et al. Nature 2020). Based on our experience with SARS-CoV-2 in hamsters, this sample size provides power to determine differences in tissue viral loads and clinical endpoints such as weight and mortality.
Data exclusions	No data were excluded.
Replication	Virologic and immunologic measures were performed in duplicate. Technical replicates were minimally different. All attempts at replication were successful.
Randomization	Animals were balanced for gender and otherwise randomly allocated to groups.
Blinding	All immunologic and virologic assays were performed blinded.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	SARS-CoV-2 virus stocks are available from BEI. Other reagents can be shared with an MTA for academic research.

Antibodies

Antibodies used	Primary rabbit anti-SARS-CoV-nucleoprotein antibody (Novus; NB100-56576 at 1:500 or 1:1000), rabbit anti-Iba-1 antibody (Wako; 019-19741 at 1:500), rabbit anti-CD3 (Dako; A0452 at 1:300), and antibodies against CD3 at 1:400 (Thermo Cat. No. RM-9107-S; clone SP7), Iba-1 at 1:500 (BioCare Cat. No. CP290A; polyclonal), or ACE-2 (Abcam; ab108252).
Validation	all mAbs used according to manufacturer's instructions and website and previously published methods; mAbs were validated and titrated for specificity prior to use

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	70 male and female Syrian golden hamsters (Envigo), 10-12 weeks old
Wild animals	None

Field-collected samples	None

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Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses

Rinke Bos[1,4], Lucy Rutten[1,4], Joan E. M. van der Lubbe[1,4], Mark J. G. Bakkers[1], Gijs Hardenberg[1], Frank Wegmann[1], David Zuijdgeest[1], Adriaan H. de Wilde[1], Annemart Koornneef[1], Annemiek Verwilligen[1], Danielle van Manen[1], Ted Kwaks[1], Ronald Vogels[1], Tim J. Dalebout[2], Sebenzile K. Myeni[2], Marjolein Kikkert[2], Eric J. Snijder[2], Zhenfeng Li[3], Dan H. Barouch[3], Jort Vellinga[1], Johannes P. M. Langedijk[1], Roland C. Zahn[1], Jerome Custers[1] and Hanneke Schuitemaker[1]✉

Development of effective preventative interventions against SARS-CoV-2, the etiologic agent of COVID-19 is urgently needed. The viral surface spike (S) protein of SARS-CoV-2 is a key target for prophylactic measures as it is critical for the viral replication cycle and the primary target of neutralizing antibodies. We evaluated design elements previously shown for other coronavirus S protein-based vaccines to be successful, e.g., prefusion-stabilizing substitutions and heterologous signal peptides, for selection of a S-based SARS-CoV-2 vaccine candidate. In vitro characterization demonstrated that the introduction of stabilizing substitutions (i.e., furin cleavage site mutations and two consecutive prolines in the hinge region of S2) increased the ratio of neutralizing versus non-neutralizing antibody binding, suggestive for a prefusion conformation of the S protein. Furthermore, the wild-type signal peptide was best suited for the correct cleavage needed for a natively folded protein. These observations translated into superior immunogenicity in mice where the Ad26 vector encoding for a membrane-bound stabilized S protein with a wild-type signal peptide elicited potent neutralizing humoral immunity and cellular immunity that was polarized towards Th1 IFN-γ. This optimized Ad26 vector-based vaccine for SARS-CoV-2, termed Ad26.COV2.S, is currently being evaluated in a phase I clinical trial (ClinicalTrials.gov Identifier: NCT04436276).

npj Vaccines (2020)5:91 ; https://doi.org/10.1038/s41541-020-00243-x

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the *Betacoronavirus* genus, the *Sarbecovirus* subgenus, and is a member of the species *SARS-related coronavirus*, and the causative agent of coronavirus disease 2019 (COVID-19)[1]. In March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak a pandemic, and the development of SARS-CoV-2 vaccines has become a global health priority.

Immune responses against the spike (S) protein are thought to be required for vaccine-elicited protection against coronaviruses. Like other class I fusion proteins, the SARS-CoV-2 S protein is intrinsically metastable[2]. In recent years, efforts have been made to stabilize various class I fusion proteins in their prefusion conformation through structure-based design. In particular, the stabilization of the so-called hinge loop that precedes the central helix (CH) was shown to be a successful approach for stabilizing a range of viral fusion glycoproteins, including respiratory syncytial virus (RSV) F[3], human immunodeficiency virus (HIV) envelope protein (Env)[4,5], Ebola glycoprotein (GP)[6], human metapneumovirus (hMPV) F[7], and Lassa GP[8]. Introduction of two consecutive proline (PP) substitutions in the S2 subunit in the hinge loop between the CH and heptad repeat 1 (HR1) stabilized the S proteins of SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV)[9,10] and recently also the SARS-CoV-2 S protein in which also the furin cleavage site present at the boundary of the S1/S2 subunits was mutated[11,12]. Several vaccine approaches using different designs of the S protein (e.g., with or without stabilizing substitutions, using a wild-type (wt) signal peptide (SP) or Tissue Plasminogen Activator (tPA) SP[13], have been described, which induce neutralizing antibodies (NAbs) and protection in animal challenge models against SARS-CoV[14], MERS-CoV[13], and SARS-CoV-2 infections[15–18]. It is assumed that an effective vaccine against coronavirus infections should induce NAbs and a Th1-skewed immune response. For SARS-CoV prototype vaccines that were generated in response to the 2003 SARS-CoV outbreak, a theoretical risk of vaccine-associated enhanced disease induction has been associated with a Th2-type immune response in animal models[19–21]. Although it is unclear whether vaccine-associated enhanced disease is a relevant concern for COVID-19 vaccines applied to humans, induction of a Th1-skewed immune response is warranted to reduce even a theoretical risk of vaccine-associated enhanced disease. Different immunogen design elements and vaccine platforms can influence these vaccine characteristics and should therefore be evaluated.

We have previously shown that vaccines based on transgenes that are delivered by recombinant replication-incompetent adenovirus type 26 vectors (Ad26) have an acceptable safety profile in humans and are able to induce neutralizing and binding antibodies, CD4 and CD8 T cell responses and a Th1-biased immune response in animals and humans[22–33]. Furthermore, the availability of industrialized and scalable manufacturing processes makes this an attractive platform for vaccine development.

[1]Janssen Vaccines & Prevention BV, Leiden, The Netherlands. [2]Molecular Virology Laboratory, Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands. [3]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA. [4]These authors contributed equally: Rinke Bos, Lucy Rutten, Joan E. M. van der Lubbe. ✉email: HSchuite@its.jnj.com

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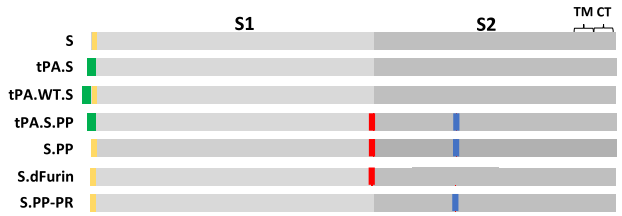

Fig. 1 S immunogen designs. Seven plasmids encoding variants of the SARS-CoV-2 S protein were produced: (1) native full-length S (S), (2) full-length S in which the wt SP is replaced by tissue plasminogen activator SP (tPA.S), (3) full-length S in which tPA SP is added upstream of the wt SP (tPA.WT.S), (4) full-length S in which SP is replaced by tPA and in which the furin cleavage site mutations and proline substitutions (K986P, V987P) have been introduced (tPA.S.PP), (5) full-length S in which furin cleavage site mutations and proline substitutions have been introduced (S.PP), (6) full-length S with only the furin cleavage site mutations (S.dFurin), and (7) full-length S with only the proline substitutions (S.PP-PR), which is therefore processed (PR) at the furin cleavage site. Green bars represent tPA SP, yellow bars represent wild-type SP, red vertical lines represent furin cleavage site mutations, and blue vertical lines represent proline substitutions.

Here we describe how we employed this vaccine platform for the development of an optimized S protein-based vaccine candidate against SARS-CoV-2.

RESULTS

SARS-CoV-2 S immunogen design

We engineered a series of plasmids coding for seven variants of the SARS-CoV-2 S protein: (1) native full-length S (S) with wt signal peptide (SP), (2) full-length S with the tissue plasminogen activator SP (tPA.S), (3) full-length S with the tPA SP positioned upstream of the wt SP (tPA.WT.S)[13], (4) full-length S with the tPA SP, the furin cleavage site mutations (R682S, R685G) and proline substitutions (K986P, V987P) (tPA.S.PP), (5) full-length S with wild-type SP, with furin cleavage site mutations and proline substitutions (S.PP), (6) full-length S with only the furin cleavage site mutations (S.dFurin), and (7) full-length S with only the proline substitutions (S.PP-PR), which can therefore be processed (PR) at the furin cleavage site in the presence of furin (Fig. 1). Constructs 6 and 7 were used to study the effects of the furin cleavage site mutations and the proline substitutions separately in vitro.

Antigenicity of SARS-CoV-2 S designs

Antigenicity of the seven membrane-bound S proteins encoded by the different DNA constructs was evaluated in cell-based ELISA (CBE). In addition, Ad26 vectors with a transgene cassette encoding five of the DNA constructs were used to transduce cells to assess transgene antigenicity by flow cytometry (Fig. 2a). Binding was assessed to four neutralizing ligands (human angiotensin-converting enzyme 2 (ACE2), convalescent human serum and two monoclonal antibodies (mAbs), S309[34] and SAD-S35 (ACROBiosystems) that bind the receptor binding domain (RBD)) and three mAbs (CR3022, CR3015, CR3046) that are non-neutralizing (Fig. S1). ACE2 and the neutralizing mAbs (1 µg/ml) showed the highest binding to the constructs that contain the double proline substitutions and/or the furin cleavage site mutations (tPA.S.PP, S.PP, SdFurin, and S.PP-PR) (Fig. 2a). ACE2 binding to these four constructs correlated with increasing ACE2 concentration, whereas ACE2 binding to the three constructs without any stabilizing substitutions showed decreased binding at the highest ACE2 concentration (5 µg/ml) (Fig. 2b), which might be caused by shedding of the S1 domain[35]. Shedding of S1 from nonstabilized S also occurs in the absence of ACE2, as ACE2 binding activity was observed in the supernatants of cell cultures transfected with construct S, but not with S.PP (Fig. S2).

The constructs containing the wt SP (S, S.PP, S.dFurin, and S.PP-PR) gave a higher ratio of neutralizing to non-neutralizing antibody binding than the tPA SP (tPA.S and tPA.S.PP) (Fig. 2c). The highest ratio of neutralizing to non-neutralizing antibody binding was observed for S.PP indicating a native prefusion conformation.

Expression of all immunogens was confirmed by western blot (Fig. 2d) using the CR3046 monoclonal antibody. Furin cleavage product S2 was only seen for those constructs with an intact furin cleavage site, confirming previous observations[36].

N-termini of mature S proteins

Correct N-terminal processing by signal peptidase is a requirement for obtaining natively folded proteins. In coronavirus S proteins, a conserved cysteine (Cys15 in case of SARS-CoV-2 S) is present directly downstream of the SP, that forms a disulfide bond with Cys136 and which is likely required for correct folding of the N-terminal domain (NTD). In silico modeling using SignalP-5.0 predicted that the wt SP would be cleaved predominantly after position 15, preventing the formation of the disulfide and leading to the presence of an unpaired Cys in the mature protein. When the wt SP was replaced by the tPA SP, the predicted cleavage occurred after position 13, allowing formation of the disulfide bond to Cys136. When a tPA SP was added upstream the wt SP, SignalP-5.0 predicted cleavage after the tPA SP. To investigate the SignalP-5.0 predictions and study the effect of different SPs on S expression and stability in more detail, we performed liquid chromatography-mass spectrometry (LC-MS/MS) to determine the N-terminus of the full-length S protein as pulled down from cell membranes using either Mab CR3022 or ACE2-Fc. In contrast to the prediction of SignalP-5.0, we found that the wt SP was cleaved after position 13, that tPA SP leads to a lower number of correct N-termini and that tPA.WT.S is cleaved predominantly after position 13 of the wt SP, but that also a small fraction of incorrectly processed signal peptide was observed with the wt SP still attached (Fig. 3).

Since the membrane-bound S protein samples gave relatively low LC-MS/MS signals, probably due to inherent complexities associated with the pull-down of membrane-anchored proteins, we sought to confirm the identity of the N-terminal residue by analyzing a pair of secreted SARS-CoV-2 S proteins that differ solely in their signal peptide. To this end we purified and characterized engineered soluble proteins that were preceded by either the wt SP or the tPA SP (S.dTM.PP and tPA.S.dTM.PP, respectively). These proteins were stabilized by furin cleavage site mutations, introduction of the two consecutive proline substitutions in the S2 hinge loop and were equipped with a T4 fibritin foldon trimerization domain. Analysis of the purified soluble proteins showed that the wt SP leads to a larger fraction of correctly cleaved N-termini compared to the tPA SP (99.5% versus 11%) (Fig. S3a). In-line with this, we observed lower trimer yields (Fig. S3c) and a lower melting temperature (Fig. S3b) for the protein with the tPA SP compared to the protein with the wt SP.

Stabilizing mutations render the S protein nonfusogenic

To further characterize our immunogen designs, we determined the effect of the furin cleavage site mutations at the S1/S2 boundary as well as the PP substitutions in the S2 hinge loop on fusion competence. To this end we developed an in vitro cell–cell fusion assay that is based on co-transfection of constructs expressing wt SARS-CoV-2 S, and variants thereof, with constructs expressing ACE2, TMPRSS2 and green fluorescent protein (GFP) in HEK293 cells (Fig. 4a). The monolayers were imaged after 24 h to visualize the syncytia through GFP redistribution. Both the dFurin

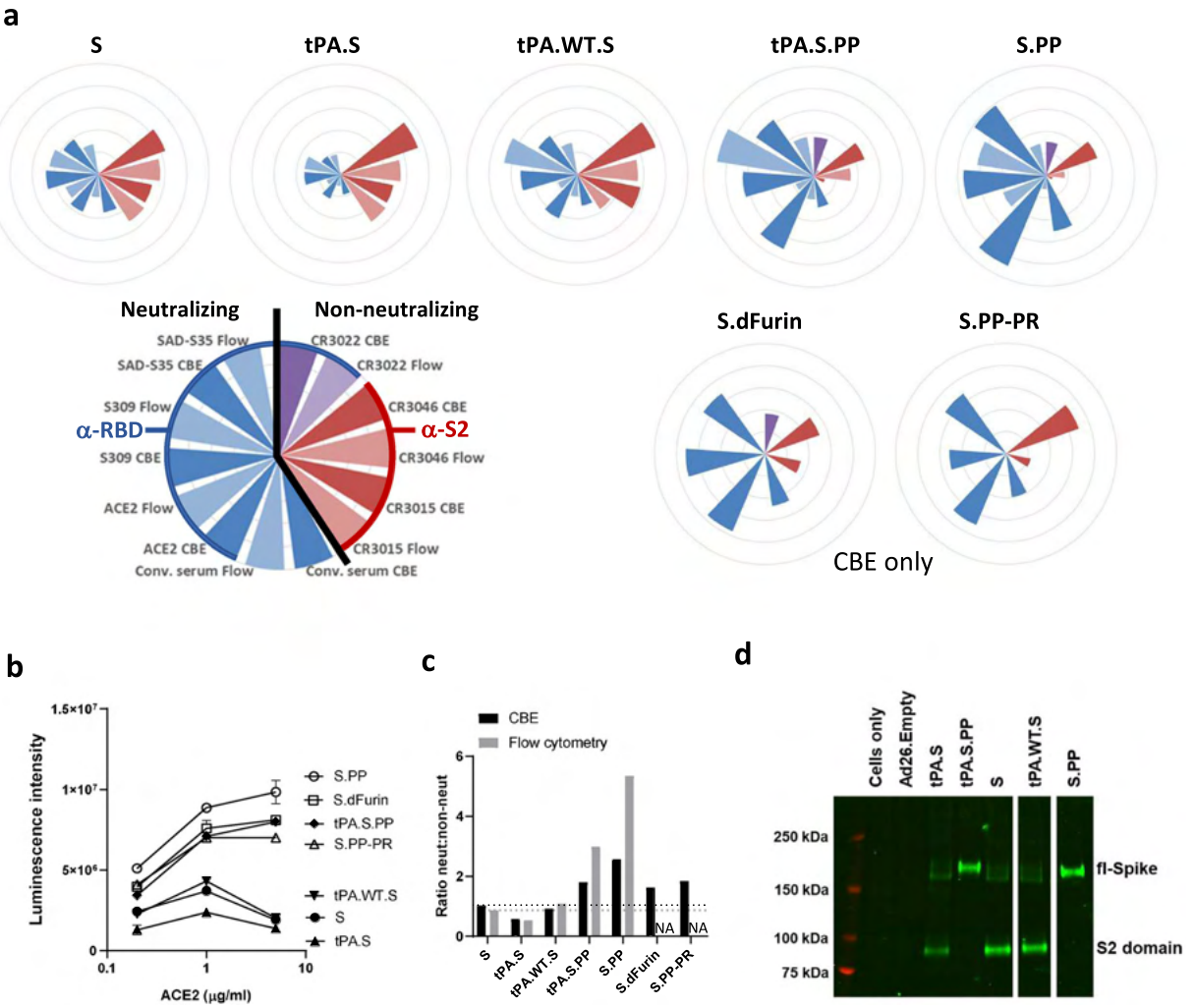

Fig. 2 S protein antigenicity. a Radar plots showing luminescence intensities measured with cell-based ELISA (CBE) and median fluorescence intensity (MFI) measured with flow cytometry ("Flow"). For CBE, cells were transfected with DNA constructs, whereas for flow cytometry cells were transduced with Ad26 vectors. Luminescence and MFI were calculated as an average of a duplicate and the MFI were normalized (by multiplying with a factor 342) to have the highest intensity the same as the highest CBE luminescence intensity. The outer ring of the circles represents a value of 10,000,000. Conv. serum stands for convalescent serum. S.dFurin and S.PP-PR were measured only with CBE. **b** ACE2 binding measured with CBE ($n = 2$). Data are represented as mean + s.d. **c** Average luminescence intensities measured with CBE or MFI measured with flow cytometry of neutralizing ligands and antibodies divided by that of non-neutralizing antibodies. The black horizontal dashed line indicates the height of the neut:non-neut ratio for S in CBE and the gray one for S in flow cytometry. **d** Western blot analysis for expression from Ad26 vaccine vectors encoding tPA.S, tPA.S.PP, S, tPA.WT.S, and S.PP in MRC-5 cell lysates under non-reduced conditions using a human monoclonal antibody (CR3046). Ad26.Empty was included as negative control.

mutations and the PP substitutions alone or in combination, were sufficient to fully prevent syncytium formation (Fig. 4b).

Next, to increase sensitivity and allow quantification of the fusion assay, we redesigned the assay using a split luciferase system. A donor cell line, expressing the S protein and one luciferase subunit, was mixed with an acceptor cell line that expresses ACE2, TMPRSS2 and the complementing luciferase subunit (Fig. 4c). In this setup, expression of the wt S protein yielded high signals that were diminished upon introduction of the PP substitutions but remained detectable (Fig. 4d). Furin cleavage site mutations reduced the signal to the background level ('no spike'). As expected, combination of both stabilizing mutations rendered the S protein nonfusogenic as well.

Stabilizing substitutions enhance in vivo immunogenicity

In parallel to the in vitro characterization, we assessed how the different construct design elements affect immunogenicity. Mice received a single intramuscular (i.m.) immunization of 10^8, 10^9, or 10^{10} viral particles (vp) of Ad26 vectors encoding different variants of the SARS-CoV-2 S protein. We observed that across doses, adeno26 with the tPA.S insert (Ad26.tPA.S) induced lower titers of antibodies that bind to the S protein than Ad26.S, as measured by ELISA at 4 and 6 weeks post immunization (Fig. 5a, b). Ad26.tPA.S.PP and Ad26.tPA.WT.S showed similar immunogenicity as Ad26.S. In addition, a live virus neutralization assay (VNA) was used to measure SARS-CoV-2 NAb responses in mice receiving 10^{10} vp (Ad26.tPA.S was not included). Ad26.tPA.S.PP did not induce higher NAb titers than Ad26.S (Fig. 5c, d), in-line with the findings described above. Ad26.tPA.WT.S induced lower NAb titers than Ad26.S albeit not statistically significant.

We next compared humoral and cellular immune responses induced by Ad26.S.PP and Ad26.S. Mice received a single i.m. immunization of 10^8, 10^9, or 10^{10} vp Ad26.S or Ad26.S.PP and binding and NAbs titers were measured at weeks 2 and 4. Both vectors elicited S protein binding antibodies in a dose-dependent

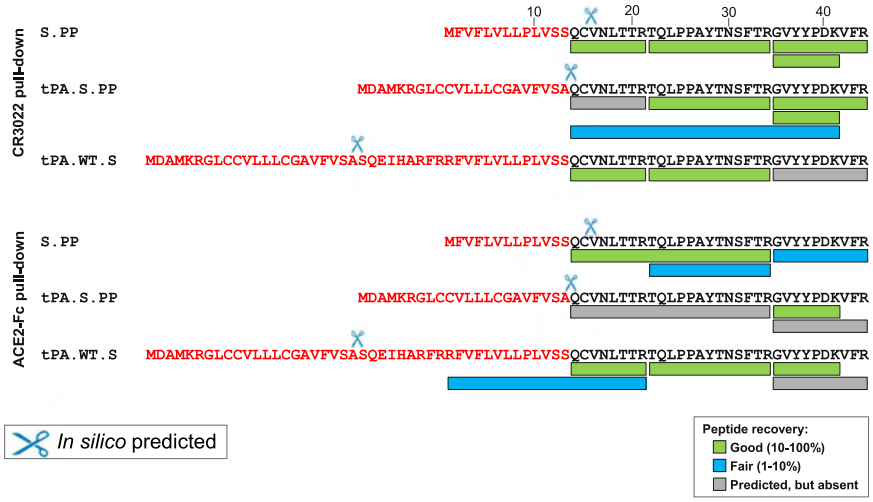

Fig. 3 Signal peptide analysis of membrane-bound S proteins. LC-MS/MS analysis of the N-terminus of S.PP, tPA.S.PP, and tPA.WT.S after either ACE2-Fc or CR3022 pull-down from cell membranes. Sequences are shown of the wt SP, tPA SP and the tPA SP linked to the wt SP (in red letters) upstream of the mature S protein sequence (in black letters). Cleavage events as predicted by SignalP-5.0 are indicated with scissors. The recovery rates of the different expected peptides are color-coded. LOD lower limit of detection.

manner (Fig. 6a, b). The induction of Nabs was only assessed for mice immunized with the highest dose (Fig. 6c, d). Titers of both binding- and NAbs were significantly higher in Ad26.S.PP immunized mice, clearly indicating the benefit of S protein stabilization for immunogenicity. These findings were consistent with immunizations with DNA vaccines encoding tPA.S, tPA.S.PP, S, and S.PP (Fig. S4).

Ad26.S.PP induces Th1-skewing of T cell response

To further investigate whether Ad26.S.PP induced a Th1-skewed immune response, to reduce the theoretical risk of vaccine-associated enhanced disease, mice were immunized i.m. with a single dose of 10^8 or 10^{10} vp Ad26.S or Ad26.S.PP and T cell responses were measured by IFN-γ ELISpot after 4 weeks. Both vaccines elicited IFN-γ producing T cells and no differences were observed between doses or vaccine constructs (Fig. 7a). To further assess the Th1/Th2 polarization by our lead vaccine candidate, mice were immunized with either Ad26.S.PP or 50 µg recombinant S protein in 100 µg aluminum phosphate adjuvant (Adjuphos), which has been shown to induce a Th2-biased immune response[37]. A single dose of Ad26.S.PP again elicited IFN-γ producing T cells as measured by ELISpot. However, recombinant S protein in Adjuphos induced undetectable or low IFN-γ ELISpot responses (Fig. 7b). Th1 dominant T cell responses were confirmed by ICS (Fig. S5). Stimulation of splenocytes with S peptide pools resulted in secretion of the Th1 hallmark cytokine IFN-γ, but secretion of typical cytokines associated with a Th2-type immune response was low. The ratios of IFN-γ concentration to either IL-4, IL-5, or IL-10 concentration was high after immunization with Ad26.S.PP suggesting that the response was Th1-skewed (Fig. 7c). S protein-specific total IgG titers were also enhanced in Ad26.S.PP compared to S protein in adjuphos immunized mice (Fig. 7d). In addition, and more important for the assessment of Th1/Th2 polarization, we measured the IgG2a/IgG1 ratio. IgG1 is produced during any type of immune response, while IgG2a is predominantly produced during a Th1-polarized immune response, and therefore an increase of the IgG2a/IgG1 ratio is indicative of the Th1-skewing of the immune response. Ad26.S.PP induced a high IgG2a/IgG1 ratio again indicative of a Th1-biased response, whereas S protein in Adjuphos induced a low IgG2a/IgG1 ratio, confirming a Th2-biased response (Fig. 7e).

DISCUSSION

According to the WHO, more than 200 COVID-19 vaccine candidates are currently in development of which most target SARS-CoV-2 S protein. Our data demonstrate that the design of the S protein is critical for the characteristics of the immunogen and the resulting immune responses elicited by the vaccine. While SARS-CoV-2-binding antibodies and NAbs have been shown to be induced in animal models and humans by several vaccines using different vaccine platforms and different S protein designs[15], we here examined different S protein designs side by side which revealed specific characteristics for each of them. We show here that the wt SP is best suited for correct cleavage, which is needed for natively folded protein and it enhanced the induction of S binding antibodies and virus NAbs in vivo. Initial in silico analysis revealed that cleavage after the wt SP could result in the removal of the structurally important cysteine at position 2 of the mature protein. However, replacement of the wt SP by the tPA SP resulted in aberrant cleavage after the conserved N-proximal cysteine, most probably required for fully correct S protein folding whereas only the wt SP resulted in fully native amino terminal cleavage. This agreed well with reduced neutralizing antibody binding to tPA.S versus S. A high ratio of neutralizing to non-neutralizing antibody binding is a qualitative measure of high expression levels of S in the prefusion conformation and/or low amount of S1 shedding[35]. The wt SP in combination with modifications that stabilize the protein in the prefusion conformation, i.e., furin cleavage site mutations and PP substitutions (S.PP), resulted in the highest ratio of neutralizing to non-neutralizing antibody binding. In vivo evaluation of the Ad26-based vaccines encoding the different S protein designs confirms that they all are immunogenic in mice after a single immunization. Induction of binding antibodies was observed for all Ad26 doses tested and S.PP was most immunogenic as it elicited the highest binding- and neutralizing antibody titers. This is likely related to the stabilizing effect of the PP substitutions and mutations in the furin cleavage site that preserve the prefusion conformation and blocks shedding of S1 (Fig. 2d). Furthermore, the combination of stabilizing mutations renders the S protein nonfusogenic, indicating that the conformational change to the post-fusion form is prevented, in-line with improved antigenicity.

An important aspect in the development of a SARS-CoV-2 vaccine is to exclude or minimize the theoretical risk of vaccine-associated enhanced disease that was observed in animal models for some of the SARS-CoV vaccine candidates[19–21]. In these

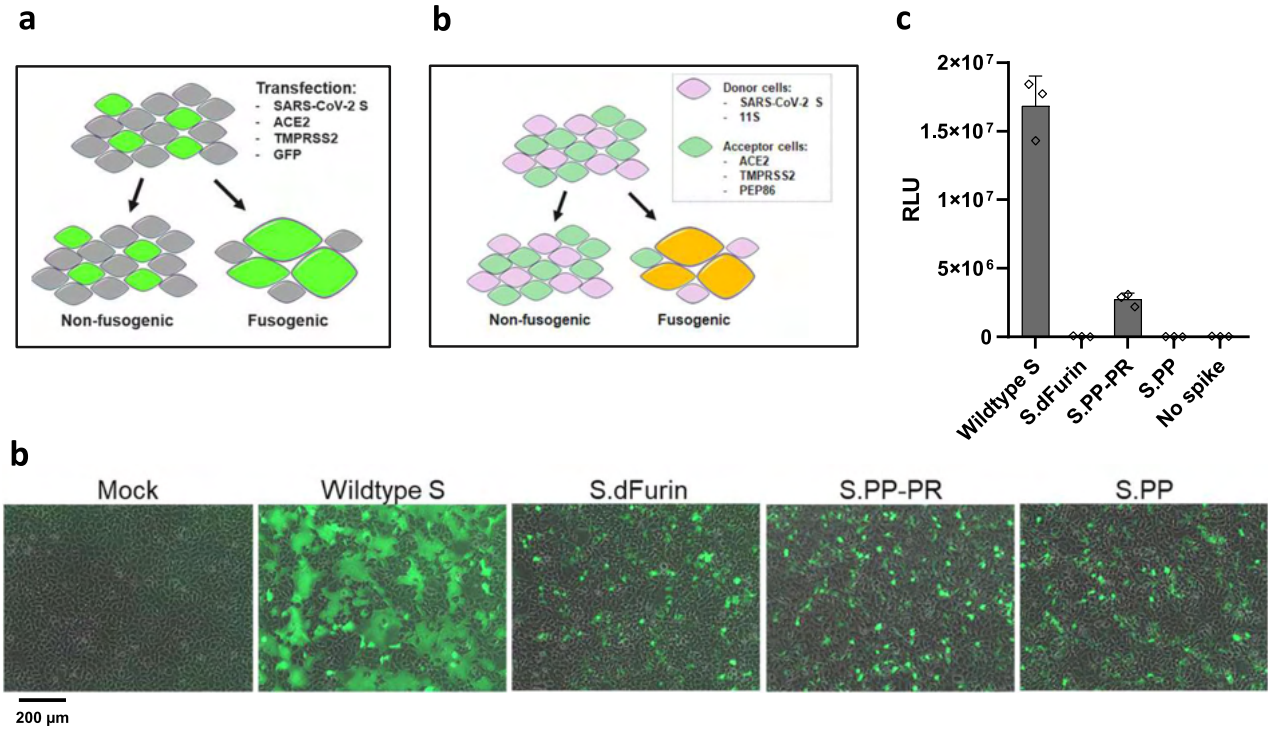

Fig. 4 Effect of stabilizing mutations on fusogenicity of S protein. a S protein fusogenicity as measured in a cell–cell fusion assay in HEK293 cells by co-transfection of plasmids encoding S protein, ACE2, TMPRSS2, and GFP. **b** Overlay of GFP and brightfield channels 24 h after transfection, as in the setup of **a**. The different S protein constructs are indicated; mock is an untransfected monolayer. The scale bar is indicating 200 µm. **c** Quantitative cell–cell fusion assay setup. **d** Luciferase signal shown as relative light units (RLU) measured at 4 h post mixing of donor and acceptor cells, as in the setup of **c**. The error bars represent one s.d.

models, vaccine-associated disease enhancement was associated with low neutralizing antibody titers and a Th2 skewed immune response. These findings, however, could not be substantiated in humans as no SARS-CoV vaccine was tested for efficacy. Genetic vaccines like Ad26 have been shown to induce a Th1-biased immune response[22–33], and we have shown here that Ad26.S.PP elicited a dominant Th1 response in combination with high titers of Nab in BALB/c mice, thereby reducing the theoretical risk of vaccine-associated enhanced disease. Ongoing animal studies are aimed to further substantiate these findings.

The potency of the Ad26-based vaccine encoding S.PP (Ad26.S.PP) in eliciting protective immunity against SARS-CoV-2 infection was successfully demonstrated in a non-human primate challenge model[36,38]. Ad26.S.PP, from now on named Ad26.COV2.S, was identified as our lead SARS-CoV-2 vaccine candidate and is currently being evaluated in a phase I clinical trial. (ClinicalTrials.gov Identifier: NCT04436276).

METHODS

Vaccine design

The S protein of SARS-CoV-2 corresponding to positions 21,536–25,384 in SARS-CoV-2 isolate Wuhan-Hu-1 (GenBank accession number: MN908947) was codon-optimized for expression in human cell lines. S designs were either based on the native SP, replacement of the native SP by the tissue plasminogen activator (tPA) SP or a SP upstream of the native signal, resulting in a sequence encoding SARS-CoV-2 amino acids 2-1273 and tPA leader as described for MERS-CoV spike protein[13,39]. In some constructs the furin cleavage site was abolished by amino acid changes R682S and R685G, or proline substitutions (K986P, V987P) were introduced.

Protein expression and purification

A plasmid encoding the SARS-CoV2 S-2P protein[12] with the wt SP and with the wt SP replaced by the tPA SP and with a C-tag for purification were codon-optimized and synthesized at GenScript. The constructs were cloned into pCDNA2004. The expression platform used was the Expi293F cells. The cells were transiently transfected using ExpiFectamine (Life Technologies) according to the manufacturer's instructions and cultured for 6 days at 37 °C and 10% CO_2. The culture supernatant was harvested and spun for 5 min at 300 × *g* to remove cells and cellular debris. The supernatant was subsequently sterile filtered using a 0.22 µm vacuum filter and stored at 4 °C until use. The C-tagged SARS-CoV2 S trimers were purified using a two-step purification protocol by 5 mL CaptureSelect™ C-tag Affinity Matrix (ThermoFisher Scientific). Proteins were further purified by size-exclusion chromatography using a HiLoad Superdex 200 16/600column (GE Healthcare).

Antibodies and reagents

SAD-S35 was purchased from ACROBiosystems (cat# SAD-S35-100ug). ACE2-Fc (ACE2) was made according to Liu et al. 2018[40]. For CR3022, CR3015[41], CR3046, and S309[34] the heavy and light chain were cloned into a single IgG1 expression vector to express a fully human IgG1 antibody. The antibodies were made by transfecting the IgG1 expression construct using the ExpiFectamine™ 293 Transfection Kit (ThermoFisher) in Expi293F (ThermoFisher) cells according to the manufacturer specifications. Antibodies were purified from serum-free culture supernatants using mAb Select SuRe resin (GE Healthcare) followed by rapid desalting using a HiPrep 26/10 Desalting column (GE Healthcare). The final formulation buffer was 20 mM NaAc, 75 mM NaCl, 5% Sucrose pH 5.5. Convalescent serum (SER-0743-00001) was obtained from Sanquin, the Netherlands.

Cell-based ELISA

HEK293 cells were seeded at 2×10^5 cells/mL in appropriate medium in a flat-bottomed 96-well microtiter plate (Corning). The plate was incubated overnight at 37 °C in 10% CO_2. After 24 h, transfection of the cells was performed with 300 ng DNA for each well and the plate was incubated for 48 h at 37 °C in 5% CO_2. Two days post transfection, cells were washed with 100 µl/well of blocking buffer containing 1% (w/v) BSA (Sigma), 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM Tris pH 8.0 in 1× PBS (GIBCO). After washing, nonspecific binding was blocked, using 100 µl/well of blocking

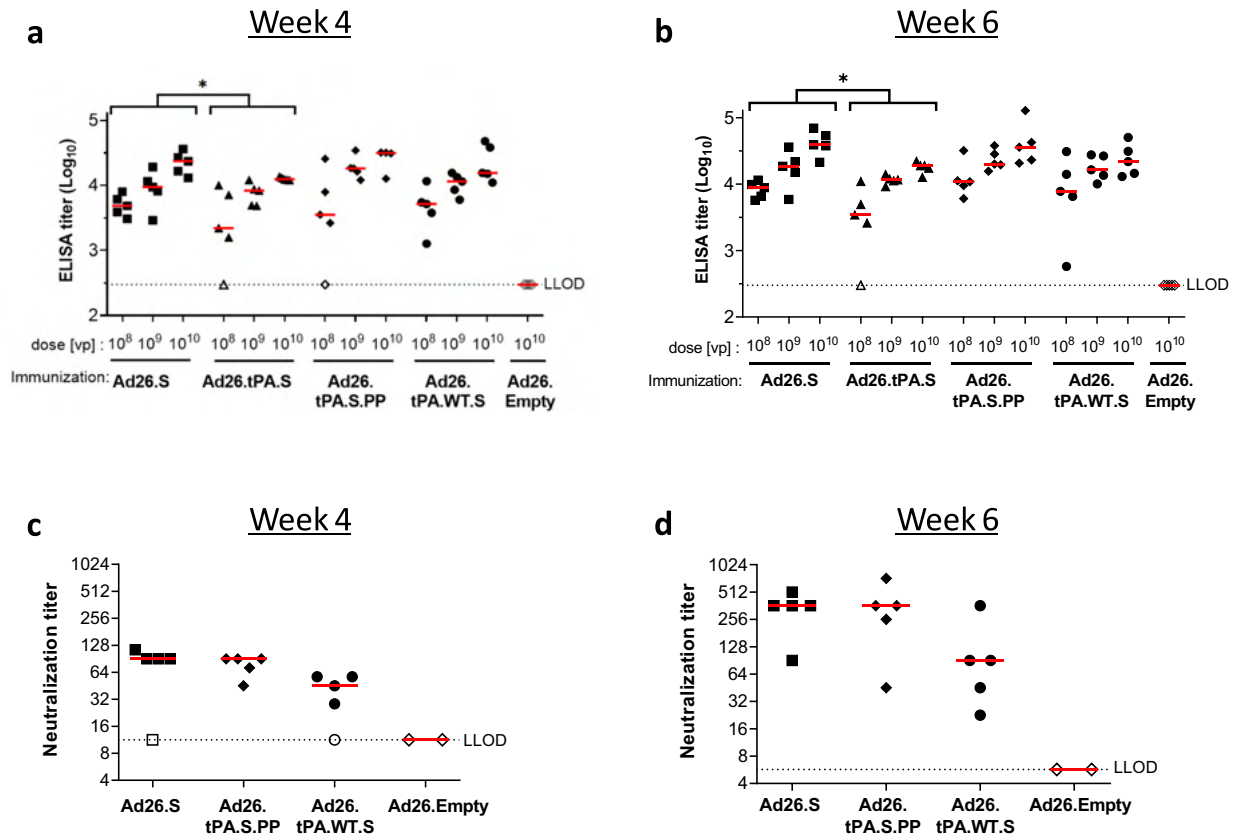

Fig. 5 Immunization with Ad26-based vaccine constructs induce humoral immune responses in immunized mice. Naïve mice (C57BL/6, $N = 5$ per group) were immunized with either 10^8, 10^9, or 10^{10} vp of Ad26-based vaccine candidates, or with 10^{10} vp of an Ad26 vector without gene insert as control (Ad26.Empty). Four weeks and six weeks after the immunization, S protein-specific binding and NAb titers were determined. **a** + **b** Spike protein-specific antibody binding titers were measured by ELISA. **c** + **d** SARS-CoV-2 NAb titers were measured by wt VNA determining the inhibition of the cytopathic effect (CPE) of virus isolate Leiden1 (L-0001) on Vero-E6 cells. Mice immunized with Ad26. Empty were taken along as two separate pools. Median responses per group are indicated with horizontal lines. Dotted lines indicate the LLOD per assay. Animals with a response at or below the LLOD were put on LLOD and are shown as open symbols. Statistical differences as determined by Cochran–Mantel–Haenszel tests (**a**, **b**) or Exact Wilcoxon rank-sum test (**c**, **d**) are indicated by asterisks; $*p < 0.05$. LLOD lower limit of detection, vp virus particles.

solution for 20 min at 4 °C. Subsequently, cells were incubated in 50 µl/well blocking buffer containing primary antibodies ACE2-Fc (5 µg/mL, 1 µg/mL and 0.2 µg/mL)(1 µg/mL for radar plot), S309 (1 µg/mL), SAD-S35 (1 µg/mL), CR3015 (5 µg/mL), CR3022 (5 µg/mL), CR3046 (5 µg/mL), and convalescent serum (1:400) for 1 hr at 4 °C. The plate was washed three times with 100 µl/well of the blocking buffer, three times with 100 µl/well of washing buffer containing 1 mM $MgCl_2$, 1.8 mM $CaCl_2$ in 1× PBS and then incubated with 100 µl/well of the blocking buffer for 5 min at 4 °C. After blocking, the cells were incubated with 50 µl/well of secondary antibodies HRP conjugated Mouse Anti-Human IgG (Jackson, 1:2500) or HRP Conjugated goat anti-mouse IgG (Jackson, 1:2500) then incubated 40 min at 4 °C. The plate was washed three times with 100 µl/well of the blocking buffer, three times with 100 µl/well washing buffer. 30 µl/well of BM Chemiluminescence ELISA substrate (Roche, 1:50) was added to the plate, and the luminosity was immediately measured using the Ensight Plate Reader.

Flow cytometry

MRC-5 cells (0.4×10^6 cells/well) were seeded in 6-well plates and after overnight growth transduced with Ad26 vectors encoding SARS-CoV-2 S transgenes at 5000 vp/cell for 48 h. Harvested cells were washed with PBS and stained with LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit (Invitrogen). For SARS-CoV-2 surface staining, cells were washed twice with PBS and then incubated with ACE2-Fc (1 µg/ml), convalescent serum (1:400), and mAbs S309, SAD-S35, CR3022, CR3015 and CR3046 (1 µg/ml) for 30 min in FACS buffer (PBS with 0.5% BSA). Cells were washed twice with FACS buffer and stained with goat anti-Human IgG Alexa Fluor 647 (Invitrogen) or goat anti-Mouse IgG Alexa Fluor 647 (Invitrogen) secondary antibody for 30 min in FACS buffer. Cells were washed twice with FACS buffer and fixed with 1× BD CellFIX (BD Biosciences) for 15 min. Cells were washed once with FACS buffer and resuspended in FACS buffer before analysis on a FACS Canto instrument (BD Biosciences). Data were analyzed with FlowJo™ Software (Becton, Dickinson and Company) and plotted as median fluorescence intensity of the MRC-5 single, live cell population (Fig. S6).

BioLayer interferometry (BLI)

Expi293F cells were transiently transfected using ExpiFectamine (Life Technologies) according to the manufacturer's instructions and cultured for 3 days at 37 °C and 10% CO2. The culture supernatant was harvested and spun for 5 min at 300 × *g* to remove cells and cellular debris. The spun supernatant was subsequently sterile filtered using a 0.22 µm vacuum filter and used as the analyte in the experiment. A solution of ACE2-Fc at a concentration of 10 µg/mL was used to immobilize the ligand on anti-hIgG (AHC) sensors (FortéBio cat#18-5060) in 1× kinetics buffer (FortéBio cat#18-1092) in 96-well black flat bottom polypropylene microplates (FortéBio cat#3694). The experiment was performed on an Octet HTX instrument (Pall-FortéBio) at 30 °C with a shaking speed of 1000 rpm. Activation was 60 s, immobilization of antibodies 600 s, followed by washing for 300 s and then binding the S proteins for 1200 s. Data analysis was performed using the FortéBio Data Analysis 8.1 software (FortéBio). Binding levels were plotted as nm shifts at 1200 s after S protein binding.

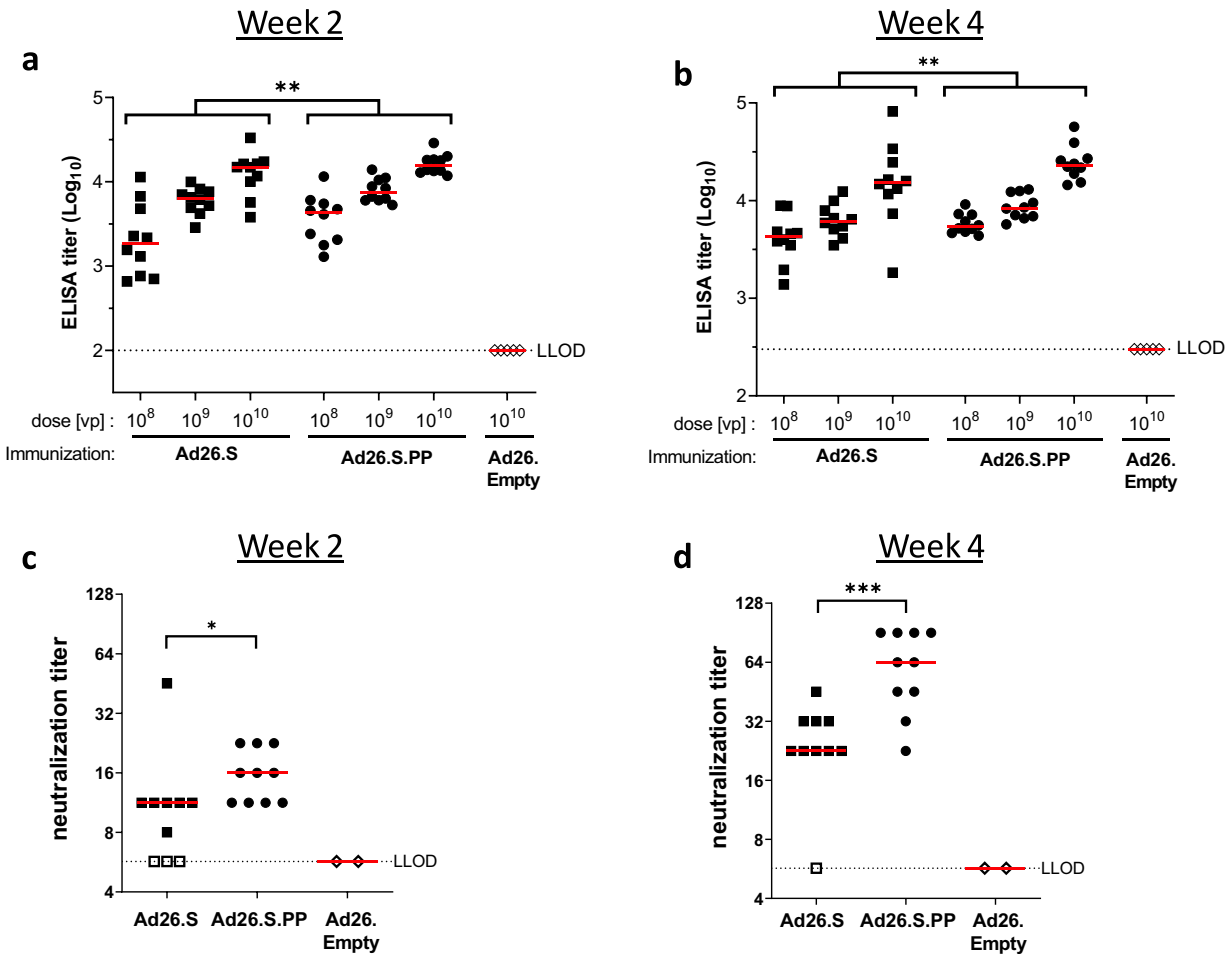

Fig. 6 Significant higher S protein binding antibody titers and SARS-CoV-2 neutralization titers induced by Ad26.S.PP compared to Ad26.S. Naïve mice (BALB/c, $N = 10$ per group) were immunized with either 10^8, 10^9, or 10^{10} vp of Ad26.S.PP, Ad26.S or with Ad26.Empty ($N = 5$). Serum was sampled 2 and 4 weeks post immunization and splenocytes were analyzed 4 weeks post immunization. **a** + **b** S protein-specific binding antibody titers were measured by ELISA. **c** + **d** SARS-CoV-2 NAb titers were measured by wt VNA. Mice immunized with Ad26.empty were taken along as two separate pools in VNA. Median responses per group are indicated with horizontal lines. Dotted lines indicate the LLOD per assay. Animals with a response at or below the LLOD were put on LLOD and are shown as open symbols. Statistically significant differences between groups per dose, or across doses (indicated by brackets), as determined by *t*-test from ANOVA, are indicated by asterisks; *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$. LLOD lower limit of detection, vp virus particles.

Differential scanning fluorometry (DSF)

0.2 mg of purified protein in 50 µl PBS pH 7.4 (Gibco) was mixed with 15 µl of 20 times diluted SYPRO orange fluorescent dye (5000 x stock, Invitrogen S6650) in a 96-well optical qPCR plate. A negative control sample containing the dye only was used for reference subtraction. The measurement was performed in a qPCR instrument (Applied Biosystems ViiA 7) using a temperature ramp from 25–95 °C with a rate of 0.015 °C per second. Data were retrieved continuously. The negative first derivative was plotted as a function of temperature. The melting temperature corresponds to the lowest point in the curve.

Mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS/MS) was used to determine the N-terminus on either purified soluble protein or on a pull-down of the full-length S protein from cell membranes using either Mab CR3022 or ACE2-Fc. The purified soluble proteins were subjected to direct digest, whereas the membrane-bound spike protein samples were either subjected to direct digest or in gel digestion. The experiments were performed on C18 nRP-LC connected to a ESI-Q-orbitrap mass spectrometer. Data processing for the different proteins was performed using Biopharma Finder 3.1 (Thermo Scientific). Each data file was compared to its corresponding amino acid sequence. Peptides were filtered by mass accuracy, confidence and structural resolution and reported. In order to pick up low abundant peptides, the thresholds for the MS noise level and the S/N were lowered 100-fold in comparison to the normal processing method.

Cell–cell fusion assay

Cell–cell fusion assays were performed to ascertain the relative fusogenicity of the different S protein variants. For fluorescent readout, full-length wild-type SARS-CoV-2 S protein and variants thereof, human ACE2, human TMPRSS2 and GFP were co-expressed from pcDNA2004 plasmids in HEK293 cells using Trans-IT transfection reagent according to the manufacturer's instructions. Transfections were performed on 70% confluent cell monolayers in 6-well plates. Transfected cells were incubated at 37 °C and 10% CO_2 for 24 h before imaging on an EVOS cell imaging system (Thermofisher). Overlays between brightfield and GFP channels were made in ImageJ.

The fluorescent fusion assay was adapted to allow quantitative measurement of cell–cell fusion by leveraging the NanoBiT complementation system (Promega). Donor HEK293 cells were transfected with full-length S and the 11 S subunit in 96-well white flat bottom TC-treated microtest assay plates. Acceptor HEK293 cells were transfected in 6-well plates (Corning) with ACE2, TMPRSS2 and the PEP86 subunit, or just the PEP86 subunit ('No spike') as negative control. Eighteen hour after transfection, the acceptor cells were released by 0.1% trypsin/EDTA and

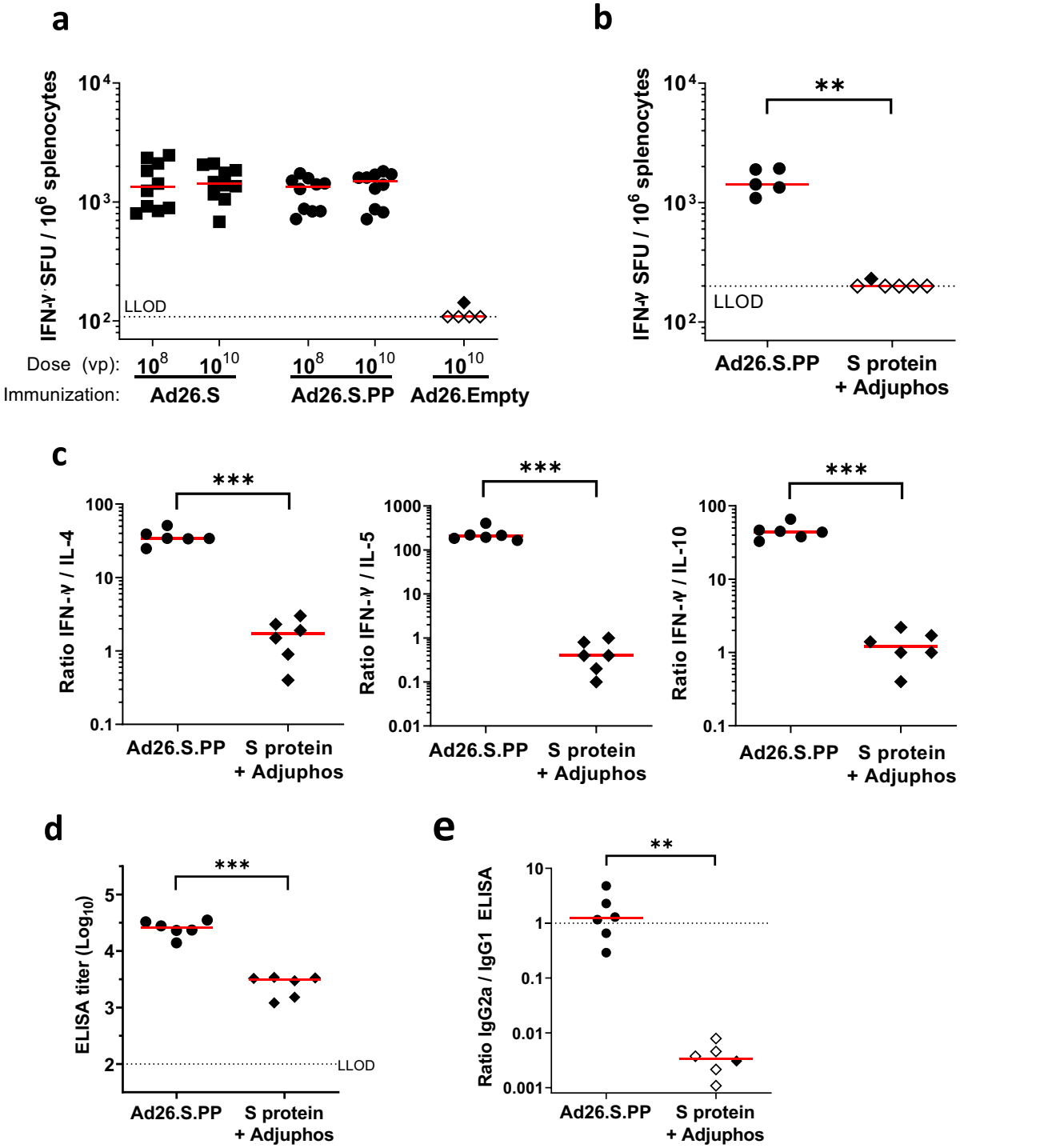

Fig. 7 Analysis of IFN-γ secretion and Th1/Th2 skewing in Ad26.S.PP immunized mice. a Naïve BALB/c mice were immunized with either 10^8 or 10^{10} vp of Ad26.S.PP, Ad26.S ($N = 10$ per group) or with Ad26.Empty ($N = 5$). Number of IFN-γ producing cells per million splenocytes 4 weeks after immunization as measured by ELISpot. Median responses per group are indicated with horizontal lines. Dotted line indicates the LLOD. Animals with a response at or below the LLOD were put on LLOD and are shown as open symbols. **b**–**e** Naïve mice (BALB/c, $N = 6$ per group) were immunized with either 10^{10} vp of Ad26.S.PP or 50 µg of S protein adjuvanted with 100 µg Adjuphos (Adju-Phos®). Two weeks after immunization samples were analyzed for antibody and cellular responses. **b** Number of IFN-γ producing cells per million splenocytes as measured by ELISpot. One mouse excluded due to high background in peptide pool 1 stimulation. Dotted line indicates the LLOD. **c** Th1 (IFN-γ) over Th2 (IL-4, IL-5, and IL-10) cytokine ratios measured by multiplex ELISA after stimulation of splenocytes with SARS-CoV-2 S protein peptides. **d** S protein-specific IgG binding antibody titers were measured by ELISA. **e** Ratio of IgG2a/IgG1 S protein-specific antibody titers as measured by IgG2a and IgG1 ELISA. Dotted line indicates an IgG2a/IgG1 ratio of 1. Animals with a response at or below the LLOD in the IgG2a ELISA are shown as open symbols. Horizontal lines denote group medians. Statistically significant differences as determined by *t*-test from ANOVA (**a**), z-test from Tobit ANOVA (**c**, **d**), or Mann–Whitney U test (**b**, **e**) are indicated by asterisks $^{**}p < 0.01$; $^{***}p < 0.001$. LLOD lower limit of detection, vp virus particles.

added to the donor cells at a 1:1 ratio for 4 h. Luciferase complementation was measured by incubating with Nano-Glo® Live Cell Reagent for 3 min, followed by readout on an Ensight plate reader (PerkinElmer).

Ad26 vectors

Replication-incompetent, E1/E3-deleted Ad26 vectors were engineered using the AdVac system[42], here using a single plasmid technology containing the Ad26 vector genome including a transgene expression cassette. The codon-optimized SARS-COV-2 Spike genes (as described in vaccine design) were inserted into the E1-position of the Ad26 vector genomes under transcriptional control of the human cytomegalovirus promoter and the SV-40 polyadenylation sequence. Rescue and manufacturing of the Ad26 vectors was performed in the complementing cell line PER.C6 TetR[43,44]. Virus particle (vp) titers in the Ad26 vector preparations were quantified by measurement of optical density at 260 nm[45] and infectivity was assessed by quantitative potency assay (QPA)[46], using PER.C6 TetR cells. PCR including subsequent sequencing of PCR products has confirmed the identity and integrity of the SARS-COV-2 Spike genes (primers used are listed in Table S1). Ad26 vector-mediated expression of SARS-COV-2 Spike genes was confirmed by western blot analysis of cell-culture lysates from infected MRC-5 cells (Fig. 2d). Bioburden levels and endotoxin levels met the preset release criteria for animal experiments.

SDS-PAGE and western blotting

Twenty-four-well plates were seeded with MRC-5 cells (1.25×10^5 cells/well), and after overnight growth transduced with Ad26 vectors encoding SARS-CoV-2 S transgenes. Cell lysates were harvested 48 h post transduction and, after heating for 5 min at 85 °C, samples were loaded under non-reduced conditions on a precast 3–8% Tris-Acetate SDS-PAGE gel (Invitrogen). Proteins were transferred to a nitrocellulose membrane using an iBlot2 dry blotting system (Invitrogen), and membrane blocking was performed overnight at 4 °C in Tris-buffered saline (TBS) containing 0.2% Tween 20 (V/V) (TBST) and 5% (W/V) Blotting-Grade Blocker (Bio-Rad). Following overnight blocking, the membrane was incubated for 1 h with 2.8 µg/ml CR3046. in TBST-5% Blocker. CR3046 is a human monoclonal antibody directed against SARS-CoV-1 Spike. After incubation, the membrane was washed three times with TBST for 5 min and subsequently incubated for 1 h with 1:10,000 IRDye 800CW-conjugated goat anti-human secondary antibody (Li-COR) in TBST-5% Blocker. Finally, the PVDF membrane was washed three times with TBST for 5 min, and after drying developed using an ODYSSEY® CLx Infrared Imaging System (Li-COR). All samples derived from one experiment and were processed in parallel. The uncropped blot is provided in Fig S7.

Animals

Animal experiments were approved by the Central Authority for Scientific Procedures on Animals (Centrale Commissie Dierproeven) and conducted in accordance with the European guidelines (EU directive on animal testing 86/609/EEC) and local Dutch legislation on animal experiments.

Female BALB/c or C57BL6 mice (specific pathogen-free), aged 8–12 weeks at the start of the study were purchased from Charles River laboratories (Sulzfeld, Germany). Mice were immunized via the intramuscular (i.m.) route with 100 µl vaccine (50 µl per hind leg) under isoflurane anesthesia. Intermediate blood samples were collected via submandibular bleeding route. At the end of the experiment, under anesthesia, animals were exsanguinated by heart puncture and sacrificed by cervical dislocation. Blood was processed for serum isolation and spleens were collected. Spleens were processed into single cell suspensions for cellular assays (when applicable).

Virus neutralization assay

Neutralization assays against live SARS-CoV-2 were performed using the microneutralization assay previously described by Algaissi and Hashem[47]. Vero-E6 cells [CRL-1580, American Type Culture Collection (ATCC)] were grown in Eagle's minimal essential medium (EMEM; Lonza) supplemented with 8% fetal calf serum (FCS; Bodinco BV), 1% penicillin-streptomycin (Sigma–Aldrich, P4458) and 2 mM L-glutamine (PAA). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Clinical isolate SARS-CoV-2/human/NLD/Leiden-0001/2020 (Leiden L-0001) was isolated from a nasopharyngeal sample and its characterization will be described elsewhere (manuscript in preparation). The NGS-derived sequence of this virus isolate is available under GenBank accession number MT705205 and shows 1 mutation in the Leiden-0001 virus compared to the Wuhan sequence resulting in Asp614 > Gly at position 614 of the Spike protein. Isolate Leiden-0001 was propagated and titrated in Vero-E6 cells using the tissue culture infective dose 50 ($TCID_{50}$) endpoint dilution method and the TCID50 was calculated by the Spearman-Kärber algorithm as described[48]. All work with live SARS-CoV-2 was performed in a biosafety level 3 facility at Leiden University Medical Center.

Vero-E6 cells were seeded at 12,000 cells/well in 96-well tissue culture plates 1 day prior to infection. Heat-inactivated (30 min at 56 °C) serum samples were analyzed in duplicate. The panel of sera were two-fold serially diluted in duplicate, with an initial dilution of 1:10 and a final dilution of 1:1280 in 60 µL EMEM medium supplemented with penicillin, streptomycin, 2 mM L-glutamine and 2% FCS. Diluted sera were mixed with equal volumes of 120 $TCID_{50}$/60 µL Leiden -0001 virus and incubated for 1 h at 37 °C. The virus-serum mixtures were then added onto Vero-E6 cell monolayers and incubated at 37 °C in a humidified atmosphere with 5% CO_2. Cells either unexposed to the virus or mixed with 120 $TCID_{50}$/60 µL SARS-CoV-2 were used as negative (uninfected) and positive (infected) controls, respectively. At 3 days post-infection, cells were fixed and inactivated with 40 µL 37% formaldehyde/PBS solution/well overnight at 4 °C. The fixative was removed from cells and the clusters were stained with 50 µL/well crystal violet solution, incubated for 10 min and rinsed with water. Dried plates were evaluated for viral cytopathic effect. Neutralization titer was calculated by dividing the number of positive wells with complete inhibition of the virus-induced cytopathogenic effect, by the number of replicates, and adding 2.5 to stabilize the calculated ratio. The neutralizing antibody titer was defined as the log2 reciprocal of this value. A SARS-CoV-2 back-titration was included with each assay run to confirm that the dose of the used inoculum was within the acceptable range of 30 to 300 $TCID_{50}$.

Pseudotyped virus neutralization assay

MLV pseudotyped with SARS-COV-2 S protein was produced as previously described[49] with some minor changes. In short, Platinum-GP cells (cell Biolabs, Inc) were transfected with a plasmid encoding the codon-optimized SARS-COV-2 Spike gene from strain Wuhan-Hu-1 (GenBank: MN908947) carrying a 19-aa cytoplasmic tail truncation, a GAG-Pol packaging vector and an MLV vector encoding a luciferase reporter gene using Lipofectamine 3000 transfection reagent (Life Technologies) according to manufacturer's protocol. Cells were incubated overnight at 37 °C 10% CO2 with OPTIMEM transfection medium. Next day, medium was replaced by OPTIMEM supplemented with 5% FBS and 1% PenStrep and incubated for 48 h prior to harvest. The harvested pseudotyped MLV particles were stored at −80 °C prior to use. In the neutralization assay, soluble ACE2-Fc and mAbs CR3015, CR3022, CR3046 and SAD-S35 (ACROBiosystems) were two-fold serial diluted ($n = 3$) in DMEM without phenol red supplemented with 1% FBS and 1% PenStrep and incubated with an equal volume of pseudotyped MLV. After 30 min incubation, the mixture was inoculated onto susceptible Vero-E6 cells in MW96 well plates. Luciferase activity was measured after 40 h of incubation by addition of an equal volume of substrate NeoLite (Perkin Elmer) followed by luminescence readout on the EnSight Multimode Plate Reader (Perkin Elmer). The percentage of infectivity was calculated as ratio of luciferase readout in the presence of mAbs normalized to luciferase readout in the absence of mAb.

Direct coat ELISAs

IgG binding to SARS-CoV-2 Spike antigen was measured by ELISA with the full-length in-house produced Spike protein COR200099. COR200099 is an in-house produced SARS-CoV-2 Spike protein, stabilized by two point mutations in the S1/S2 junction that knocks out the furin cleavage site, and by two newly introduced prolines in the hinge region in S2. In addition, the transmembrane and cytoplasmic regions have been replaced by a foldon domain for trimerization mutations, allowing the protein to be produced as soluble protein (see S.dTM.PP Fig. 3C). Briefly, 96-wells Perkin Elmer white ½ area plates were O/N coated with COR200099 protein. Next day plates were washed, blocked for 1 h and subsequently incubated for 1 h with 3-fold serially diluted serum samples in block buffer in duplicate. After washing, plates were incubated for 1 h with goat anti-mouse IgG-HRP in block buffer, washed again and developed using ECL substrate. Luminescence readout was performed using a BioTek Synergy Neo instrument.

For IgG1 and IgG2a ELISAs, a similar protocol was followed as described above, but respectively using Goat anti-Mouse IgG1-HRP and Goat anti-Mouse IgG2a-HRP as secondary antibodies.

ELISpot assay

IFN-γ ELISpot was performed on splenocytes of mice isolated after sacrifice using mouse IFN-γ ELISpot-plus kit (Mabtech). Splenocytes were obtained by disaggregation of spleens with the gentleMACS dissociator. IFN-γ ELISpot assay was performed by stimulating splenocytes from individual mice for 18 h with two different peptide pools (pool 1; peptides 1-156, and pool 2; peptides 157-313) consisting of in total 313 15-mers peptides overlapping by 11 amino acids together covering the full-length Spike protein at a final concentration of 1 µg/peptide/mL. Results shared in this manuscript are the sum of stimulation with peptide pool 1 and pool 2. PMA/ionomycin stimulation was used as a positive control (data not shown); medium was used as negative control and used to calculate the lower limit of detection. Stimulation was done overnight in duplicate wells containing 0.5–2.5 × 105 cells per well.

Multiplex ELISA

The Th1/Th2 multiplex ELISA assay was performed on splenocytes obtained after sacrifice. Splenocytes were stimulated by 48 h culturing in the presence of two Spike 15-mer peptide pools (pool 1 and pool 2). Resulting supernatants were diluted 4-fold and measured for the presence of Th1 (IFN-γ) and Th2 cytokines (IL-10, IL-4, and IL-5) using a 10-plex multiplex ELISA proinflammatory panel (mouse) kit (Meso Scale Discovery, V-PLEX Proinflammatory Panel 1 Mouse Kit cat# K15048D). Results shared in this manuscript are the sum of stimulation with peptide pool 1 and pool 2. Ratios of Th1/Th2 cytokines (IFN-γ/IL-10, IFN-γ/IL-4, and IFN-γ/IL-5) were calculated on basis of cytokine measurements in supernatants by dividing the Th1 cytokine (IFN-γ) with the respective Th2 cytokines. Multiplex ELISA measurements were done on supernatant diluted either 2-fold or 4-fold.

ICS assay

The intracellular cytokine staining assay (ICS) was performed on splenocytes obtained two weeks after sacrifice. Splenocytes were obtained by disaggregation of spleens with the gentleMACS dissociator. ICS assay was performed by stimulating splenocytes from individual mice for 16 h, 10^6 cells per well, with two different peptide pools (pool 1; peptides 1-156, and pool 2; peptides 157-313) consisting of in total 313 15-mers peptides overlapping by 11 amino acids together covering the full-length Spike protein at a final concentration of 0.2 µg/peptide/well. Stimulation with peptide pools was done at 37 °C, 10% CO_2 for 1 h in presence of Rat-anti-Mouse CD49d (1:500, BD; cat #553153) and Hamster-anti-Mouse CD28 (1:500, BD; cat#553294). Protein transport was blocked (1:1000; BD GolgiPlug™ cat#51-230KZ) overnight at 37 °C, 10% CO_2. Cells were washed twice with PBS and stained during 20 min at room temperature in the dark, according to manufacturers' instructions, with a violet viability dye (1:5000; Invitrogen Violet ViD cat#L34955). Cells were washed twice with 0.5% BSA in PBS (PBA) and Fc receptors were blocked during 10 min in the fridge in the dark (1:50; BD Mouse Fc block cat# 553142). Cells were washed once with PBA and stained during 30 min in the fridge in the dark with anti-CD3e FITC (BD, cat#553062), anti-CD4 PerCP-Cy5.5 (BD, cat#550954) and anti-CD8α APC-H7 (BD, cat#557654). Cells were washed twice with PBA and permeabilized/fixated during 20 min in the fridge in the dark (BD Cytofix/Cytoperm™ cat#51-2090KZ/554722), after which 1× BD Perm/Wash™ buffer (BD cat#51-2091KZ/554723) was added. Cells were stained during 30 mins in the fridge, in the dark with anti-IFN-γ PE (BD, cat#554412), anti-TNFa PE-Cy7 (557644) and anti-IL-2 APC (BD, cat#554429). Cells were washed twice with Perm/Wash buffer, resuspended in PBA, and fluorescence was measured on the BD FACSCanto™ II and analyzed with BD FACSDiva™ software: Flow Jo version 10.06.1. Cells were gated on single cells, excluding dead cells, and gated for lymphocytes (Fig S8a). CD8-$CD4^+$ and $CD8^+$ T cells were then gated on IFN-γ, IL-2, and TNF-α (Fig S8b).

Statistical analysis

Statistical differences between immunization regimens were evaluated two-sided for S-specific binding antibodies as measured by ELISA, NAb titers as measured by VNA, IFN-γ producing cells as measured by ELISpot and cytokine production by MSD and ICS assays. Comparisons between Ad26.S, Ad26.tPA.S, Ad26.tPA.SS, Ad26.tPA.WT.S, Ad26.S.PP, adjuvanted S protein, tPA.S, tPA.S.PP, S and S.PP groups were made using the exact Wilcoxon rank-sum test, Cochran–Mantel–Haenszel test, Mann–Whitney U test, *t*-test from ANOVA, or z-test from Tobit ANOVA. Results were corrected for multiple comparisons by Bonferroni correction; 3-fold Bonferroni correction Fig. 5a, b, 2-fold Bonferroni correction Fig. 5c, d.

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc. Cary, NC, US) and R version 3.6.1 (2019-07-05). Statistical tests were conducted two-sided at an overall significance level of $\alpha = 0.05$.

Reporting summary

Further information on research design is available in the Nature Research Life Sciences Reporting Summary linked to this article.

DATA AVAILABILITY

All data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Coronaviridae Study Group of the International Committee on Taxonomy of, V. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.* **5**, 536–544 (2020).
2. Sternberg, A. & Naujokat, C. Structural features of coronavirus SARS-CoV-2 spike protein: targets for vaccination. *Life Sci.* **257**, 118056 (2020).
3. Krarup, A. et al. A highly stable prefusion RSV F vaccine derived from structural analysis of the fusion mechanism. *Nat. Commun.* **6**, 8143 (2015).
4. Rutten, L. et al. A universal approach to optimize the folding and stability of prefusion-closed HIV-1 envelope trimers. *Cell Rep.* **23**, 584–595 (2018).
5. Sanders, R. W. et al. Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. *J. Virol.* **76**, 8875–8889 (2002).
6. Rutten, L. et al. Structure-based design of prefusion-stabilized filovirus glycoprotein trimers. *Cell Rep.* **30**, 4540–4550 (2020). e4543.
7. Battles, M. B. et al. Structure and immunogenicity of pre-fusion-stabilized human metapneumovirus F glycoprotein. *Nat. Commun.* **8**, 1528 (2017).
8. Hastie, K. M. et al. Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923–928 (2017).
9. Kirchdoerfer, R. N. et al. Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. *Sci. Rep.* **8**, 15701 (2018).
10. Pallesen, J. et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proc. Natl Acad. Sci. USA* **114**, E7348–E7357 (2017).
11. Walls, A. C. et al. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* **181**, 281–292 (2020). e286.
12. Wrapp, D. et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260–1263 (2020).
13. Alharbi, N. K. et al. ChAdOx1 and MVA based vaccine candidates against MERS-CoV elicit neutralising antibodies and cellular immune responses in mice. *Vaccine* **35**, 3780–3788 (2017).
14. Martin, J. E. et al. A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial. *Vaccine* **26**, 6338–6343 (2008).
15. Corbett, K. S. et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature* https://doi.org/10.1038/s41586-020-2622-0 (2020).
16. Smith, T. R. F. et al. Immunogenicity of a DNA vaccine candidate for COVID-19. *Nat. Commun.* **11**, 2601 (2020).
17. van Doremalen, N. et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature* https://doi.org/10.1038/s41586-020-2608-y (2020).
18. Yu, J. et al. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science* https://doi.org/10.1126/science.abc6284 (2020).
19. Honda-Okubo, Y. et al. Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic immunopathology. *J. Virol.* **89**, 2995–3007 (2015).
20. Iwata-Yoshikawa, N. et al. 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. *J. Virol.* **88**, 8597–8614 (2014).
21. Tseng, C. T. et al. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. *PLoS ONE* **7**, e35421 (2012).

22. Anywaine, Z. et al. Safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Uganda and Tanzania. *J. Infect. Dis.* **220**, 46–56 (2019).
23. Baden, L. R. et al. Assessment of the safety and immunogenicity of 2 novel vaccine platforms for HIV-1 prevention: a randomized trial. *Ann. Intern. Med.* **164**, 313–322 (2016).
24. Baden, L. R. et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). *J. Infect. Dis.* **207**, 240–247 (2013).
25. Mutua, G. et al. Safety and immunogenicity of a 2-dose heterologous vaccine regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Nairobi, Kenya. *J. Infect. Dis.* **220**, 57–67 (2019).
26. Shukarev, G., Callendret, B., Luhn, K., Douoguih, M. & consortium, E. A two-dose heterologous prime-boost vaccine regimen eliciting sustained immune responses to Ebola Zaire could support a preventive strategy for future outbreaks. *Hum. Vaccin Immunother.* **13**, 266–270 (2017).
27. Winslow, R. L. et al. Immune responses to novel adenovirus type 26 and modified vaccinia virus Ankara-vectored Ebola vaccines at 1 year. *JAMA* **317**, 1075–1077 (2017).
28. Barouch, D. H. et al. Characterization of humoral and cellular immune responses elicited by a recombinant adenovirus serotype 26 HIV-1 Env vaccine in healthy adults (IPCAVD 001). *J. Infect. Dis.* **207**, 248–256 (2013).
29. Barouch, D. H. et al. Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). *Lancet* **392**, 232–243 (2018).
30. Liu, J. et al. Magnitude and phenotype of cellular immune responses elicited by recombinant adenovirus vectors and heterologous prime-boost regimens in rhesus monkeys. *J. Virol.* **82**, 4844–4852 (2008).
31. Milligan, I. D. et al. Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia Ankara-vectored Ebola vaccines: a randomized clinical trial. *JAMA* **315**, 1610–1623 (2016).
32. Radosevic, K. et al. The Th1 immune response to Plasmodium falciparum circumsporozoite protein is boosted by adenovirus vectors 35 and 26 with a homologous insert. *Clin. Vaccin. Immunol.* **17**, 1687–1694 (2010).
33. van der Fits, L. et al. Adenovector 26 encoded prefusion conformation stabilized RSV-F protein induces long-lasting Th1-biased immunity in neonatal mice. *npj Vaccines* **5**, 49 (2020).
34. Pinto, D. et al. Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290–295 (2020).
35. Tortorici, M. A. & Veesler, D. Structural insights into coronavirus entry. *Adv. Virus Res.* **105**, 93–116 (2019).
36. Mercado, N. B. et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature* https://doi.org/10.1038/s41586-020-2607-z (2020).
37. Lindblad, E. B. Aluminium compounds for use in vaccines. *Immunol. Cell Biol.* **82**, 497–505 (2004).
38. Chandrashekar, A. et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. *Science* https://doi.org/10.1126/science.abc4776 (2020).
39. Gilbert, S. C., Hill, A. V. S. & Moris, S. J. Compositions and methods for inducing an immune response. patent WO 2018/215766 Al (2017).
40. Liu, P. et al. Novel ACE2-Fc chimeric fusion provides long-lasting hypertension control and organ protection in mouse models of systemic renin angiotensin system activation. *Kidney Int.* **94**, 114–125 (2018).
41. van den Brink, E. N. et al. Molecular and biological characterization of human monoclonal antibodies binding to the spike and nucleocapsid proteins of severe acute respiratory syndrome coronavirus. *J. Virol.* **79**, 1635–1644 (2005).
42. Abbink, P. et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J. Virol.* **81**, 4654–4663 (2007).
43. Wunderlich, K. et al. Potent and short promoter for expression of heterologous genes. patent WO/2018/146205 (2018).
44. Zahn, R. et al. Ad35 and ad26 vaccine vectors induce potent and cross-reactive antibody and T-cell responses to multiple filovirus species. *PLoS ONE* **7**, e44115 (2012).
45. Maizel, J. V. Jr., White, D. O. & Scharff, M. D. The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A, and 12. *Virology* **36**, 115–125 (1968).
46. Wang, F. et al. Using QPCR to assign infectious potencies to adenovirus based vaccines and vectors for gene therapy: toward a universal method for the facile quantitation of virus and vector potency. *Vaccine* **23**, 4500–4508 (2005).
47. Algaissi, A. & Hashem, A. M. Evaluation of MERS-CoV neutralizing antibodies in sera using live virus microneutralization assay. *Methods Mol. Biol.* **2099**, 107–116 (2020).
48. Hierholzer, J. C. & Killington, R. A. in *Virology Methods Manual* 1st edn. 25–46 (Academic Press, 1996).
49. Millet, J. K. & Whittaker, G. R. Murine Leukemia Virus (MLV)-based coronavirus Spike-pseudotyped particle production and infection. *Bio Protoc.* https://doi.org/10.21769/BioProtoc.2035 (2016).

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AUTHOR CONTRIBUTIONS

Designed studies and reviewed data: R.B., L.R., J.E.M.vd.L., M.J.G.B., G.H., F.W., D.Z., R.V., A.H.d.W., M.K., E.J.S., S.K.M., J.V., J.P.M.L., R.Z., J.C., A.V., A.K., D.v.M., J.C., T.K., H.S. Design of vaccines: L.R., M.J.G.B., R.B, A.H.d.W., D.Z., T.K., J.P.M.L., F.W., D.v.M., Z.L., R.C.Z., J.V., J. C., R.V., D.H.B., H.S. Performed experiments: T.J.D., S.K.M., A.K. Drafted the paper: all authors.

COMPETING INTERESTS

The authors declare no competing financial interests. R.B., L.R., M.J.G.B., F.W., D.Z., and J.P.L. are co-inventors on related vaccine patents. R.B., L.R., J.E.M.vd.L., M.J.G.B., G.H., F. W., D.Z., A.H.d.W., A.K., A.V., D.v.M., T.K., R.V., J.V., J.P.M.L., R.C.Z, J.C., and H.S. are employees of Janssen Vaccines & Prevention BV. R.B., L.R., F.W., D.Z., D.v.M., T.K., R.V., J. V., J.P.M.L., R.C.Z, J.C., and H.S. hold stock of Johnson & Johnson.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to H.S.

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ORIGINAL ARTICLE

Interim Results of a Phase 1–2a Trial of Ad26.COV2.S Covid-19 Vaccine

J. Sadoff, M. Le Gars, G. Shukarev, D. Heerwegh, C. Truyers, A.M. de Groot, J. Stoop, S. Tete, W. Van Damme, I. Leroux-Roels, P.-J. Berghmans, M. Kimmel, P. Van Damme, J. de Hoon, W. Smith, K.E. Stephenson, S.C. De Rosa, K.W. Cohen, M.J. McElrath, E. Cormier, G. Scheper, D.H. Barouch, J. Hendriks, F. Struyf, M. Douoguih, J. Van Hoof, and H. Schuitemaker

ABSTRACT

BACKGROUND

Efficacious vaccines are urgently needed to contain the ongoing coronavirus disease 2019 (Covid-19) pandemic of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A candidate vaccine, Ad26.COV2.S, is a recombinant, replication-incompetent adenovirus serotype 26 (Ad26) vector encoding a full-length and stabilized SARS-CoV-2 spike protein.

METHODS

In this multicenter, placebo-controlled, phase 1–2a trial, we randomly assigned healthy adults between the ages of 18 and 55 years (cohort 1) and those 65 years of age or older (cohort 3) to receive the Ad26.COV2.S vaccine at a dose of 5×10^{10} viral particles (low dose) or 1×10^{11} viral particles (high dose) per milliliter or placebo in a single-dose or two-dose schedule. Longer-term data comparing a single-dose regimen with a two-dose regimen are being collected in cohort 2; those results are not reported here. The primary end points were the safety and reactogenicity of each dose schedule.

RESULTS

After the administration of the first vaccine dose in 805 participants in cohorts 1 and 3 and after the second dose in cohort 1, the most frequent solicited adverse events were fatigue, headache, myalgia, and injection-site pain. The most frequent systemic adverse event was fever. Systemic adverse events were less common in cohort 3 than in cohort 1 and in those who received the low vaccine dose than in those who received the high dose. Reactogenicity was lower after the second dose. Neutralizing-antibody titers against wild-type virus were detected in 90% or more of all participants on day 29 after the first vaccine dose (geometric mean titer [GMT], 224 to 354), regardless of vaccine dose or age group, and reached 100% by day 57 with a further increase in titers (GMT, 288 to 488) in cohort 1a. Titers remained stable until at least day 71. A second dose provided an increase in the titer by a factor of 2.6 to 2.9 (GMT, 827 to 1266). Spike-binding antibody responses were similar to neutralizing-antibody responses. On day 14, CD4+ T-cell responses were detected in 76 to 83% of the participants in cohort 1 and in 60 to 67% of those in cohort 3, with a clear skewing toward type 1 helper T cells. CD8+ T-cell responses were robust overall but lower in cohort 3.

CONCLUSIONS

The safety and immunogenicity profiles of Ad26.COV2.S support further development of this vaccine candidate. (Funded by Johnson & Johnson and the Biomedical Advanced Research and Development Authority of the Department of Health and Human Services; COV1001 ClinicalTrials.gov number, NCT04436276.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Schuitemaker at Janssen Vaccines and Prevention, Newtonweg 1, Leiden 2301CA, the Netherlands, or at hschuite@its.jnj.com.

Drs. Sadoff and Le Gars contributed equally to this article.

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The ongoing coronavirus disease 2019 (Covid-19) pandemic that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1,2] has affected millions of people globally. To contribute to the containment of this pandemic — and to stop the pressure on health care systems and the negative effects on the global economy — efficacious Covid-19 vaccines are urgently needed.[3-6]

One of the candidate vaccines, Ad26.COV2.S, is a recombinant, replication-incompetent adenovirus serotype 26 (Ad26) vector encoding a full-length and stabilized SARS-CoV-2 spike (S) protein.[7,8] The vaccine was derived from the first clinical isolate of the Wuhan strain (Wuhan 2019; whole genome sequence, NC_045512). The Ad26 vector is used in the Ebola vaccine that was approved by the European Medicines Agency and in vaccine candidates against respiratory syncytial virus, human immunodeficiency virus, and Zika virus.[9-11] Ad26-based vaccines are generally safe and highly immunogenic.[9-11] Here, we report the interim results of a multicenter, randomized, double-blind, placebo-controlled, phase 1–2a clinical trial (COV1001) involving healthy adults in two age cohorts to evaluate the safety, reactogenicity, and immunogenicity of Ad26.COV2.S.

Methods

Trial Design and Participants

The trial was initiated on July 22, 2020, at 12 centers in Belgium and the United States. Trial participants included healthy adults between the ages of 18 and 55 years and those 65 years of age or older. The younger group was divided into cohort 1a (with a target enrollment of 375 participants) and cohort 1b (an exploratory cohort for in-depth analysis of immunogenicity, with a target enrollment of 25 participants). The older age group was included in cohort 3, with a target enrollment of 375 participants. In November 2020, enrollment was initiated in cohort 2 to collect longer-term data comparing a single-dose regimen with a two-dose regimen (as described in the Supplementary Appendix, available with the full text of this article at NEJM.org). Only the interim results in cohorts 1 and 3 are reported here. All the participants provided written informed consent before enrollment.

Participants in cohorts 1 and 3 received Ad26.COV2.S at a dose of either 5×10^{10} viral particles (low dose) or 1×10^{11} viral particles (high dose) per milliliter, administered intramuscularly in a single-dose or two-dose schedule 56 days apart. The trial design called for an evaluation of the boosting effect of Ad26.COV2.S at 6 months and 1 year after vaccination with respect to safety, reactogenicity, and immunogenicity in each cohort. Additional details regarding the trial design are provided in Table S1 and Fig. S1 in the Supplementary Appendix and in the protocol, also available at NEJM.org.

Trial Oversight

The trial was reviewed and approved by the local ethics committee or institutional review board at each site. Janssen Vaccines and Prevention, one of the Janssen Pharmaceuticals companies acquired by Johnson & Johnson, was the regulatory sponsor of the trial and holder of the Investigational New Drug application. The trial was funded by Johnson & Johnson and the Biomedical Advanced Research and Development Authority of the Department of Health and Human Services. Janssen representatives designed and manufactured the vaccine candidate, designed the trial, developed the statistical analysis plan, and performed the analyses. The decision to submit the manuscript for publication was made by all authors, who vouch for the accuracy and completeness of the reported data and for the fidelity of the trial to the protocol. No one who is not an author contributed to the writing of the manuscript.

Procedures

In cohorts 1 and 3, we randomly assigned the participants in a 1:1:1:1:1 ratio to one of five vaccination groups: low dose followed by low dose, low dose followed by placebo, high dose followed by high dose, high dose followed by placebo, and placebo followed by placebo (Fig. S1). Data that are reported here were collected after the administration of the second dose (either vaccine or placebo) in cohort 1a and after the first dose in cohort 3. Randomization was performed by means of an interactive Web-response system and stratified according to site with the use of randomly permuted blocks. Participants and investigators remained unaware of trial-group assignments throughout the trial. To meet the criteria for blinding, the sponsor and statisticians were informed about group assignments for the primary analysis of results in cohorts 1 and 3 after 8 days

had elapsed since the administration of the second vaccine dose in all the participants.

PRIMARY AND SECONDARY END POINTS

The primary end points were the safety and reactogenicity of each dose schedule. Follow-up visits to evaluate reactogenicity, safety, and immunogenicity were scheduled on days 7, 28, and 71 after vaccination in each cohort. We collected data regarding solicited adverse events from patients' diary cards for 7 days after vaccination, data regarding unsolicited adverse events for 28 days after vaccination, and data regarding serious adverse events throughout the course of the trial after each vaccination. Adverse events were graded according to the guidance document Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials of the Food and Drug Administration. Safety data are included up to the cutoff date of October 30, 2020, in cohorts 1 and 3. The secondary end point was humoral and cellular immunity to the SARS-CoV-2 S protein.

LABORATORY ANALYSES

We used an enzyme-linked immunosorbent assay (ELISA) to measure SARS-CoV-2 S-specific binding antibodies at baseline and on days 15, 29, 57, and 71. Seropositivity was defined as a titer above the lower limit of quantitation of the assay (50.3 EU per milliliter). Clinicians at Public Health England measured SARS-CoV-2 serum neutralizing-antibody titers in a random subgroup of samples by means of a wild-type virus microneutralization assay using the Victoria/1/2020 SARS-CoV-2 strain, with seropositivity defined as a 50% maximal inhibitory concentration (IC_{50}) titer of more than 58 at the lower limit of quantitation. IC_{80} titers on wild-type virus microneutralization assay were also assessed at these time points. S-specific T-cell responses were measured at baseline and on day 15 by intracellular cytokine staining with the use of two pools of S-peptide pools of 15 mers overlapping by 11 amino acids. In CD4+ T cells, a response in type 1 helper T (Th1) cells was characterized by the expression of interferon-γ, interleukin-2, or both and not interleukin-4, interleukin-5, or interleukin-13; a response in type 2 helper T (Th2) cells was characterized by the expression of interleukin-4, interleukin-5, or interleukin-13 (or all three cytokines) plus CD40L. All assays were conducted in a blinded fashion and are described in detail in the Supplementary Appendix.

STATISTICAL ANALYSIS

We determined that the enrollment of approximately 805 participants would provide a descriptive safety and immunogenicity assessment. We first enrolled 15 participants (3 per vaccination group) in cohort 1a; after the review of safety data for the sentinel enrollees, we proceeded to full enrollment of cohort 1a. The same process was used in the enrollment of participants in cohort 3. We used log-transformed data to calculate the confidence intervals of the geometric means. Details regarding the statistical analysis plan are provided in the Supplementary Appendix and in the protocol.

RESULTS

PARTICIPANTS

From July 22 to August 7, 2020, a total of 593 persons underwent screening for enrollment in cohort 1 (including 1a and 1b combined) (Fig. S1). Of these persons, 405 were enrolled and 402 received the first dose of Ad26.COV2.S; these participants had received the second dose by November 7, 2020. From August 3 to August 24, 2020, a total of 660 persons underwent screening for cohort 3. Of these participants, 405 were enrolled and 403 received the first dose of Ad26.COV2.S. (Details regarding age distribution are provided in Table S2.) Analyses of data obtained from participants in cohort 3 after the administration of the second dose, as well as durability and longer-term safety data, are ongoing.

At baseline, the percentage of participants who were seropositive for SARS-CoV-2 S-specific antibodies was 2% in cohort 1a and 1% in cohort 3. The baseline characteristics of the participants were broadly similar across the groups (Table 1).

VACCINE SAFETY AND REACTOGENICITY

Data regarding both solicited and unsolicited adverse events and serious adverse events were available for more than 99% of the participants who returned diary cards. The investigator's assessment of reactogenicity after the administration of the first dose of vaccine was available for 402 participants in cohort 1 and for 403 participants in cohort 3. In the two cohorts, solicited local adverse events were mostly of grade 1 or 2; the

Table 1. Characteristics of the Participants at Baseline.*

Characteristic	Low-Dose Vaccine Group	High-Dose Vaccine Group	Placebo Group	All Participants
Cohort 1 (ages 18–55 yr)†				
No. of participants	162	158	82	402
Sex — no. (%)				
Male	78 (48)	72 (46)	40 (49)	190 (47)
Female	84 (52)	85 (54)	42 (51)	211 (52)
Nonbinary	0	1 (1)	0	1 (<1)
Age — yr				
Mean	36.1±10.1	34.8±10.3	35.4±10.0	35.4±10.2
Range	18–55	19–55	19–55	18–55
Race or ethnic group — no. (%)‡				
White	149 (92)	145 (92)	70 (85)	364 (91)
Black	4 (2)	7 (4)	9 (11)	20 (5)
Asian	5 (3)	5 (3)	0	10 (2)
Native Hawaiian or other Pacific Islander	1 (1)	0	0	1 (<1)
American Indian or Alaska Native	3 (2)	0	0	3 (1)
Hispanic or Latino	8 (5)	5 (3)	4 (5)	17 (4)
Multiple	0	1 (1)	0	1 (<1)
Unknown	0	0	3 (4)	3 (1)
Body-mass index§	24.5±3.3	24.6±3.1	24.5±3.0	24.6±3.2
SARS-CoV-2 seropositive — no. (%)¶	3 (2)	2 (1)	2 (2)	7 (2)
Cohort 3 (age ≥65 yr)				
No. of participants	161	161	81	403
Sex — no. (%)				
Male	84 (52)	79 (49)	38 (47)	201 (50)
Female	77 (48)	82 (51)	43 (53)	202 (50)
Age — yr				
Mean	69.6±4.0	70.0±4.2	69.9±3.7	69.8±4.0
Range	65–83	65–88	65–79	65–88
Race or ethnic group — no. (%)				
White	158 (98)	158 (98)	81 (100)	397 (99)
Black	1 (1)	2 (1)	0	3 (1)
American Indian or Alaska Native	1 (1)	0	0	1 (<1)
Hispanic or Latino	1 (1)	2 (1)	3 (4)	6 (1)
Unknown	1 (1)	0	0	1 (<1)
Not reported	0	1 (1)	0	1 (<1)
Body-mass index§	25.3±2.8	25.5±2.7	25.2±3.1	25.4±2.8
SARS-CoV-2 seropositive — no. (%)	1 (1)	2 (1)	1 (1)	4 (1)

* Plus–minus values are means ±SD. The participants in cohorts 1 and 3 received Ad26.COV2.S at a dose of either 5×10^{10} viral particles (low-dose group) or 1×10^{11} viral particles (high-dose group) in a 1-ml volume. The participants were grouped according to pooled groups (low dose followed by low dose together with low dose followed by placebo, high dose followed by high dose together with high dose followed by placebo, and placebo followed by placebo).
† Cohort 1 includes both cohorts 1a and 1b.
‡ Race or ethnic group was reported by the participants, who could report more than one category.
§ The body-mass index is the weight in kilograms divided by the square of the height in meters. This calculation was based on the weight and height measured at the time of screening.
¶ Only seronegative participants were enrolled in cohort 1b, according to the protocol.

most frequent event was injection-site pain. In cohort 1, solicited local adverse events were reported in 103 of 162 low-dose recipients (64%), in 123 of 158 high-dose recipients (78%), and in 7 of 82 placebo recipients (9%) (Fig. 1A and Table S3). In cohort 3, solicited local adverse events were reported in 66 of 161 low-dose recipients (41%), in 68 of 161 high-dose recipients (42%), and in 11 of 81 placebo recipients (14%) (Fig. 1B).

In the two cohorts, most solicited systemic adverse events were of grade 1 or 2; the most frequent events were fatigue, headache, and myalgia. In cohort 1, solicited systemic adverse events were reported in 105 low-dose recipients (65%), in 133 high-dose recipients (84%), and in 21 placebo recipients (26%). In cohort 3, solicited systemic adverse events were reported in 74 low-dose recipients (46%), in 88 high-dose recipients (55%), and in 19 placebo recipients (23%).

In cohort 1, solicited grade 3 systemic adverse events were reported in 15 low-dose recipients (9%) and in 32 high-dose recipients (20%); no placebo recipients reported such events. In cohort 1a, among the participants between the ages of 18 and 30 years who had one or more solicited grade 3 adverse events, 24% had received the low dose and 26% had received the high dose; in those between the ages of 31 and 45 years, the corresponding percentages were 43% and 14%; and in those between the ages of 46 and 55 years, the corresponding percentages were 3% and 11%. In cohort 3, grade 3 solicited systemic adverse events were reported in 1 low-dose recipient (1%) and in 4 high-dose recipients (2%); no placebo recipients reported having such events.

In cohort 1, fever was reported in 25 low-dose recipients (15%) and in 62 high-dose recipients (39%); grade 3 fever (temperature range, 39.0 to 40.0°C) was reported in 8 low-dose recipients (5%) and in 15 high-dose recipients (9%). In cohort 3, fever was reported in 7 low-dose recipients (4%) and in 14 high-dose recipients (9%); grade 3 fever was reported in no low-dose recipients and in 2 high-dose recipients (1%). No participants in the placebo group in either cohort reported having fever. All cases of fever occurred within 2 days after immunization and resolved within 1 or 2 days; more than 80% of the participants with fever received an antipyretic drug at the onset of symptoms.

In cohort 1, unsolicited adverse events were reported in 34 low-dose recipients (21%), in 56 high-dose recipients (35%), and in 14 placebo recipients (17%). In cohort 3, unsolicited adverse events were reported in 27 low-dose recipients (17%), in 38 high-dose recipients (24%), and in 13 placebo recipients (16%) (Table S4). No grade 4 adverse events (solicited or unsolicited) were reported in any cohort.

In cohort 1a, safety data after the administration of the second dose of vaccine were available for 363 participants (Fig. S2). One or more solicited adverse events were noted in 77% and 80% of the participants in the low-dose and high-dose groups, respectively, as compared with 34% and 31% of those who received placebo as a second dose after a first dose of vaccine and in 22% of those who received placebo for both doses. Solicited adverse events of grade 3 or higher were noted in 1% of low-dose recipients and in 7% of high-dose recipients; the corresponding percentages were 1% and 2% among participants in the placebo group who received a first dose of vaccine and in no participants who received placebo for both doses. No grade 3 fevers were reported in any group after a second dose of vaccine.

No participant discontinued the trial because of an adverse event. Five serious adverse events occurred: one case of hypotension that was deemed by the investigator to be unrelated to the vaccine because of a history of recurrent hypotension; one case of bilateral nephrolithiasis in a participant with a history of kidney stones (not related); one case of legionella pneumonia (not related); one worsening of multiple sclerosis, which had remained undiagnosed for approximately 8 to 10 years on the basis of findings on magnetic resonance imaging (not related); and one case of fever that resulted in hospitalization because of suspicion of Covid-19. In the last case, the participant recovered within 12 hours, and the fever was subsequently deemed by the investigator to be related to the vaccine. Details regarding all safety data are provided in the Supplementary Appendix.

IMMUNOGENICITY AND SEROCONVERSION

Immunogenicity data for this interim analysis were unblinded according to dose level. In all five groups in cohort 1a, the binding-antibody geometric mean concentration (GMC), as reported in ELISA units per milliliter, was measured against

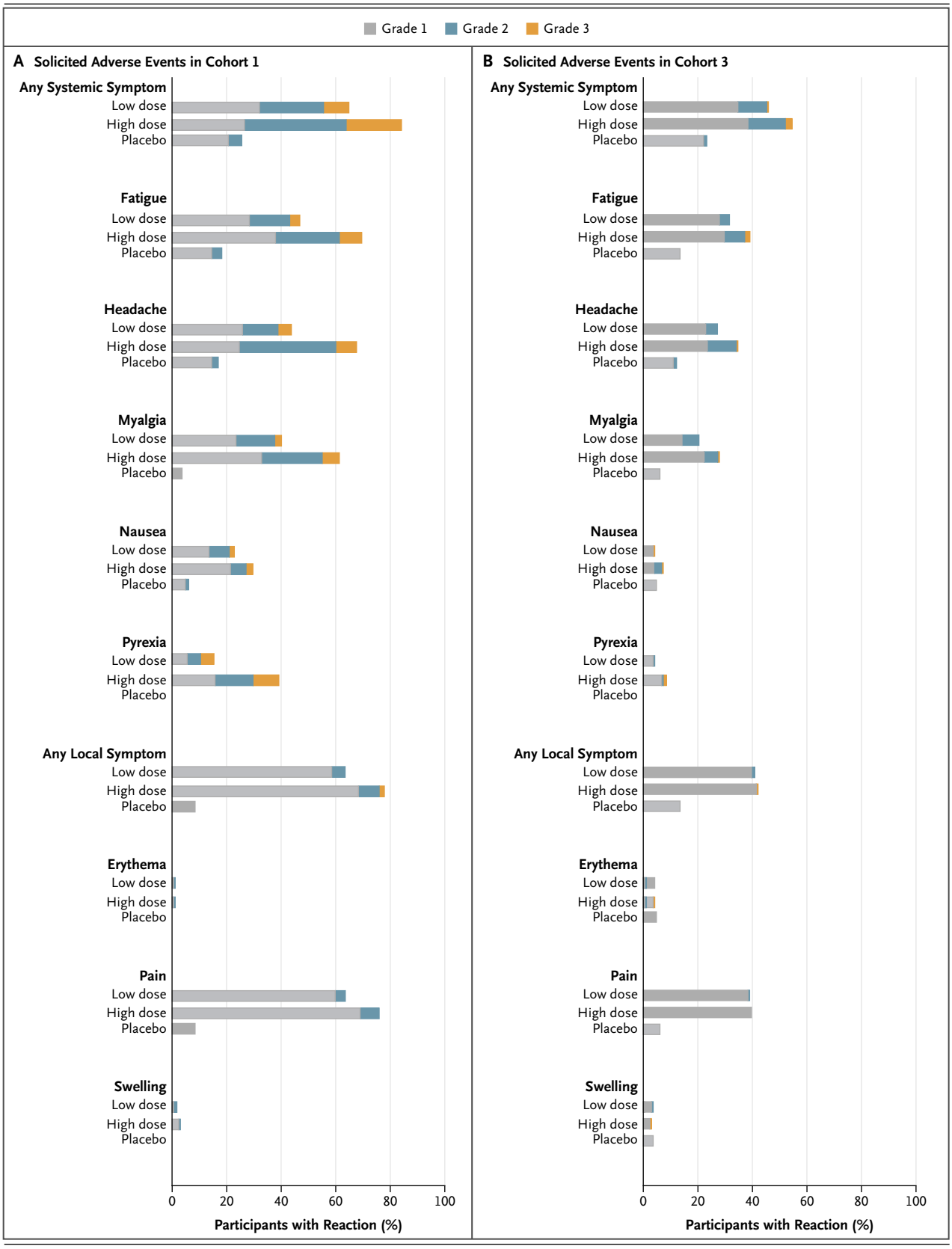


Figure 1 (facing page). Solicited Adverse Events in Cohorts 1 and 3 after the First Vaccine Dose.

Shown are solicited adverse events in participants who received the Ad26.COV2.S vaccine at a dose of 5×10^{10} viral particles (low dose) or 1×10^{11} viral particles (high dose) per milliliter or placebo. Healthy adults between the ages of 18 and 55 years were included in cohort 1 (Panel A), and those 65 years of age or older were included in cohort 3 (Panel B). The younger group was divided into cohorts 1a and 1b, with the latter designated as an exploratory cohort for in-depth analysis of immunogenicity. As shown here, data for cohorts 1a and 1b have been pooled. Data for patients in cohort 1a who received a second dose of vaccine are provided in Figure S2 in the Supplementary Appendix.

a stabilized SARS-CoV-2 full-length spike protein. At baseline, the GMC values in all the participants were lower than the lower limit of quantitation. By day 29 after vaccination, the values had increased to 478 (95% confidence interval [CI], 379 to 603) in the low-dose/placebo group, 586 (95% CI, 445 to 771) in the low-dose/low-dose group, 625 (95% CI, 505 to 773) in the high-dose/placebo group, and 788 (95% CI, 628 to 988) in the high-dose/high-dose group, with an incidence of seroconversion of 99% or more in all the groups (Fig. 2A and Fig. S3A). By day 57, the corresponding GMC values had further increased to 660 (95% CI, 513 to 849), 754 (95% CI, 592 to 961), 873 (95% CI, 701 to 1087), and 1100 (95% CI, 908 to 1332). After the first dose, the incidence of seroconversion was 100% in all but the high-dose/placebo group (97%). Fourteen days after the second dose, the GMC was 1677 (95% CI, 1334 to 2109) in the low-dose/low-dose group and 2292 (95% CI, 1846 to 2845) in the high-dose/high-dose group, with 100% seroconversion in each group. On day 71, in the low-dose/placebo and high-dose/placebo groups, the GMC was 600 (95% CI, 443 to 814) and 951 (95% CI, 696 to 1,300), respectively, values that were similar to those on day 57.

In cohort 3, the GMCs in all the participants were also below the lower limit of quantitation at baseline. By day 15 after vaccination, the GMC had increased to 122 (95% CI, 97 to 152) in the low-dose group and to 141 (95% CI, 114 to 175) in the high-dose group, with a seroconversion incidence of 75% and 77%, respectively. By day 29, the GMC was 312 (95% CI, 246 to 396) in the low-dose group and 350 (95% CI, 281 to 429) in the high-dose group, with 96% seroconversion.

The SARS-CoV-2 neutralizing-antibody titer (IC_{50}) was measured in a random subgroup of participants in cohorts 1a and 3. In cohort 1a, the geometric mean titer (GMT) was below the lower limit of quantitation at baseline and by day 29 after vaccination had increased to 224 (95% CI, 158 to 318) in the low-dose/placebo group, 224 (95% CI, 168 to 298) in the low-dose/low-dose group, 215 (95% CI, 169 to 273) in the high-dose/placebo group, and 354 (95% CI, 220 to 571) in the high-dose/high-dose group, with an incidence of seroconversion of 96%, 88%, 96%, and 92%, respectively (Fig. 2B and Fig. S3B). By day 57, the GMT had further increased to 310 (95% CI, 228 to 422), 288 (95% CI, 221 to 376), 370 (95% CI, 268 to 511), and 488 (95% CI, 334 to 714), respectively, with a 100% incidence of seroconversion in the low-dose/placebo group and 96% seroconversion in the other groups.

In cohort 1a, 14 days after the second dose, the GMT was 827 (95% CI, 508 to 1183) in the low-dose/low-dose group and 1266 (95% CI, 746 to 2169) in the high-dose/high-dose group, with 100% seroconversion in the two dose groups. On day 71, the GMT was 321 (95% CI, 227 to 438) in the low-dose/placebo group and 388 (95% CI, 290 to 509) in the high-dose/placebo group, values that were similar to those on day 57; the incidence of seroconversion was 100% in both groups.

In cohort 3, the GMTs in all the participants were below the lower limit of quantitation at baseline and had increased to 212 (95% CI, 137 to 284) in the low-dose group and 172 (95% CI, 119 to 269) in the high-dose group on day 15 and to 277 (95% CI, 193 to 307) and 212 (95% CI, 163 to 266), respectively, on day 29. The incidence of seroconversion was 91% and 84%, respectively, on day 15 and 96% and 88%, respectively, on day 29. These data were confirmed on IC_{80} analysis (Fig. S4).

Antibody levels as measured on wild-type virus neutralization assay and ELISA were strongly correlated in the two cohorts (Fig. S5). However, the correlation had a wider elliptical shape in cohort 3, which suggested more variability in the relationship between the neutralizing-antibody titer and the binding-antibody titer in the older adults. Antibody levels in the different human convalescent serum panels that were included in assays for humoral-immunity assessment that were performed in different laboratories and in serum samples that were obtained from vaccine recipi-

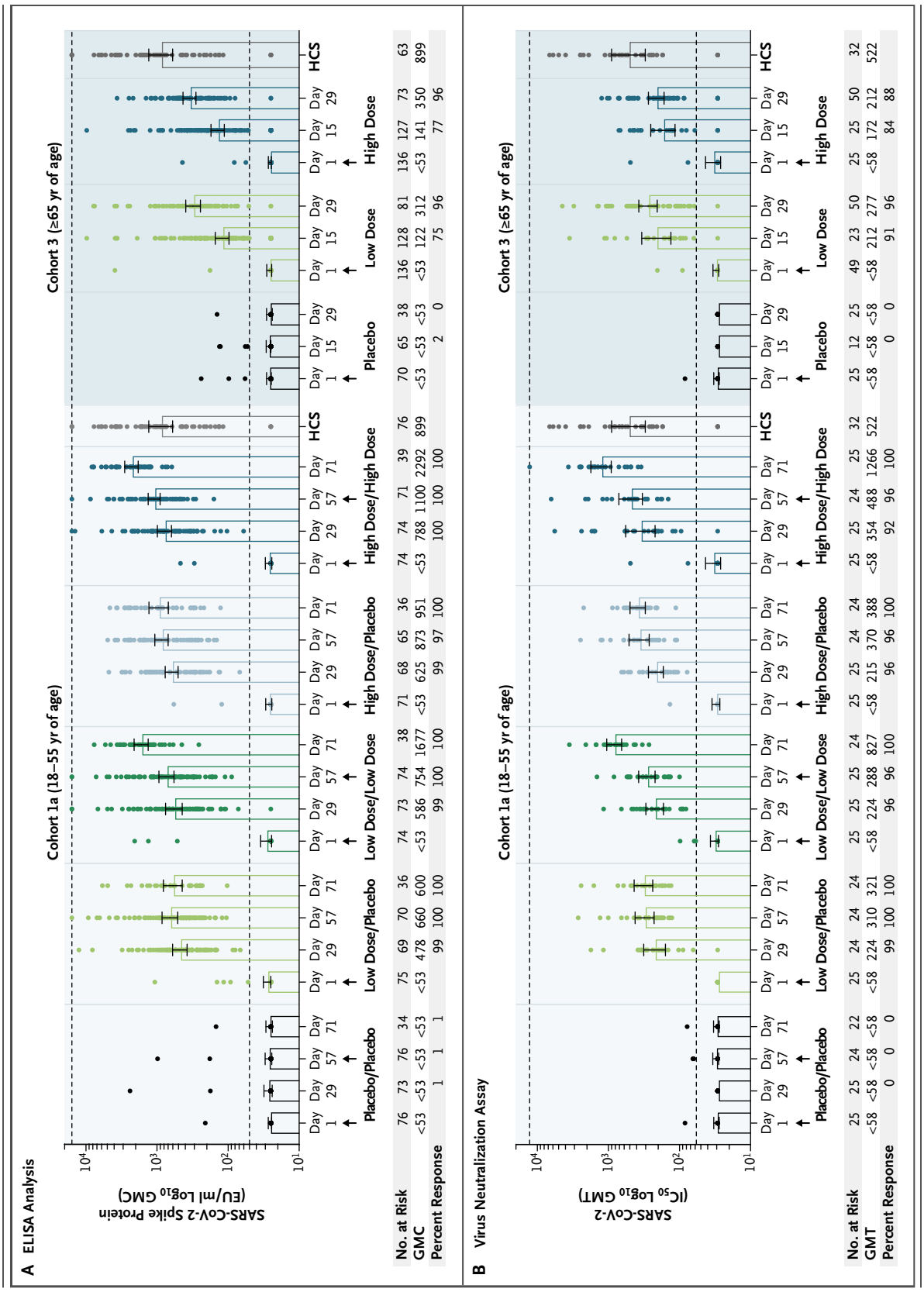


Figure 2 (facing page). Humoral Immunogenicity.
Shown are measures of humoral immunogenicity in serum samples obtained from the participants in cohort 1a (left side) and cohort 3 (right side), according to the receipt of the low or high dose of Ad26.COV2.S or placebo. In cohort 1a, the participants received two injections of high-dose or low-dose vaccine or placebo, as indicated with slashes (e.g., placebo/placebo if they received two injections of placebo). The samples were measured on enzyme-linked immunosorbent assay (ELISA) in ELISA units (EU) per milliliter (Panel A) and on wild-type virus neutralization assay, with seropositivity defined as a half maximal inhibitory concentration (IC_{50}) titer of more than 58 at the lower limit of quantitation (Panel B). Logarithmic values are reported as the geometric mean concentration (GMC) in the ELISA analyses and as the geometric mean titer (GMT) in the neutralizing-antibody analyses. The values were measured at baseline and at day 29 after vaccination in all the participants and on days 57 and 71 in those in cohort 1a. The two horizontal dotted lines in each panel indicate the lower and upper limits of quantitation of the respective assay; values below the lower line have been imputed to half the lower limit of quantitation. I bars indicate 95% confidence intervals. HCS denotes human convalescent serum.

ents were in the same range. Details regarding differences in values according to demographic characteristics are provided in Tables S5 and S6 in the Supplementary Appendix. Levels of Ad26 neutralizing antibodies at baseline or after the first dose of vaccine did not correlate with the levels of SARS-CoV-2 neutralizing antibodies on either day 29 or day 71 (Fig. S6).

S-SPECIFIC T-CELL RESPONSES

The vaccine-elicited responses in S-specific CD4+ Th1 and Th2 cells and in CD8+ T cells were assessed in a subgroup of participants at baseline and 15 days after the first dose. In cohort 1a, a Th1 response to S peptides was detected in 76% (95% CI, 65 to 86) of low-dose recipients and in 83% (95% CI, 73 to 91) of high-dose recipients; the corresponding values in cohort 3 were 60% (95% CI, 46 to 74) and 67% (95% CI, 53 to 79), respectively (Fig. 3A). In cohort 1a, the median CD4+ Th1 response to S peptides increased from an undetectable level at baseline to a median of 0.08% (interquartile range [IQR], 0.05 to 0.16) in low-dose recipients and 0.11% (IQR, 0.07 to 0.16) in high-dose recipients on day 15; in cohort 3, the corresponding values were 0.09% (IQR, 0.04 to 0.17) and 0.11% (IQR, 0.04 to 0.15), respectively. A low-dose recipient in cohort 1a and a high-dose recipient in cohort 3 had a measurable Th2 response (Fig. 3B). However, all the participants who had a measurable Th1 or Th2 response had a Th1:Th2 ratio that was well above 1, which indicated a vaccine-induced Th1-skewed response.

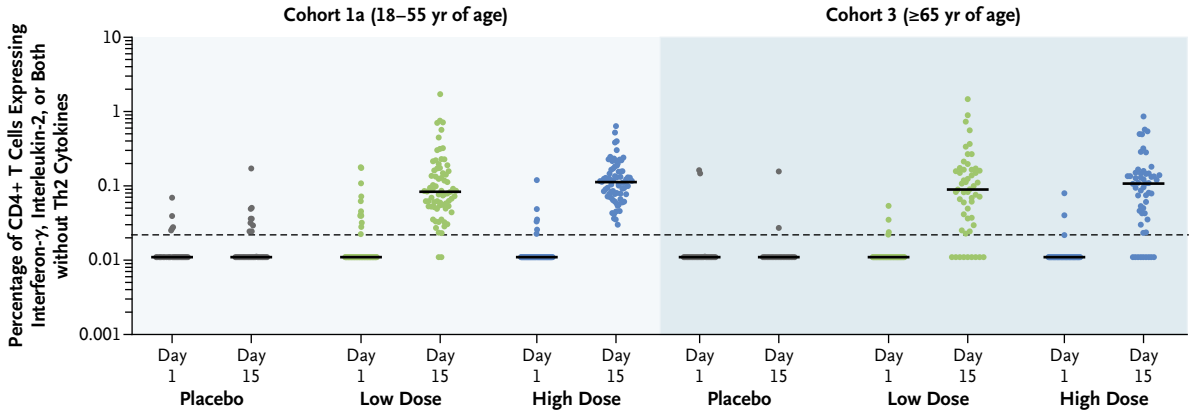
S-specific CD8+ T-cell responses, as identified by the expression of interferon-γ or interleukin-2 cytokines on S-peptide stimulation, were absent at baseline in the two cohorts (Fig. 3C). On day 15 in cohort 1a, a CD8+ T-cell response was detected in 51% of participants (95% CI, 39 to 63) in the low-dose group and in 64% (95% CI, 52 to 75) in the high-dose group, with a median S-specific CD8+ T-cell response of 0.07% (IQR, 0.03 to 0.19) and 0.09% (IQR, 0.05 to 0.19), respectively. In cohort 3, CD8+ T-cell responses were lower, with an incidence of 36% (95% CI, 23 to 51) in the low-dose group and 24% (95% CI, 13 to 37) in the high-dose group, with a median response of 0.06% (IQR, 0.02 to 0.12) and 0.02% (IQR, 0.01 to 0.08), respectively. The correlation between CD4+ Th1 and CD8+ T-cell response was poor in the two cohorts (Fig. S7).

DISCUSSION

The interim analysis of our phase 1–2a trial showed that the Ad26.COV2.S vaccine had an acceptable safety and reactogenicity profile and was immunogenic after a single vaccination with either the low or high dose. After the administration of the first dose, a trend toward a higher incidence of solicited systemic adverse events was noted with the higher vaccine dose, and a clear trend for decreasing grade 3 adverse events with increasing age was observed. The local and systemic reactions occurred on the day of immunization or the next day and generally resolved within 24 hours. The systemic reactions were very responsive to antipyretic drugs, and no need for the prophylactic use of such drugs was identified. After the second dose among participants between the ages of 18 and 55 years, the incidence of grade 3 solicited systemic adverse events was much lower than that after the first immunization in both the low-dose and high-dose groups, a finding that contrasts with observations with respect to messenger RNA–based vaccines, for which the second dose has been associated with increased reactogenicity.[4,6]

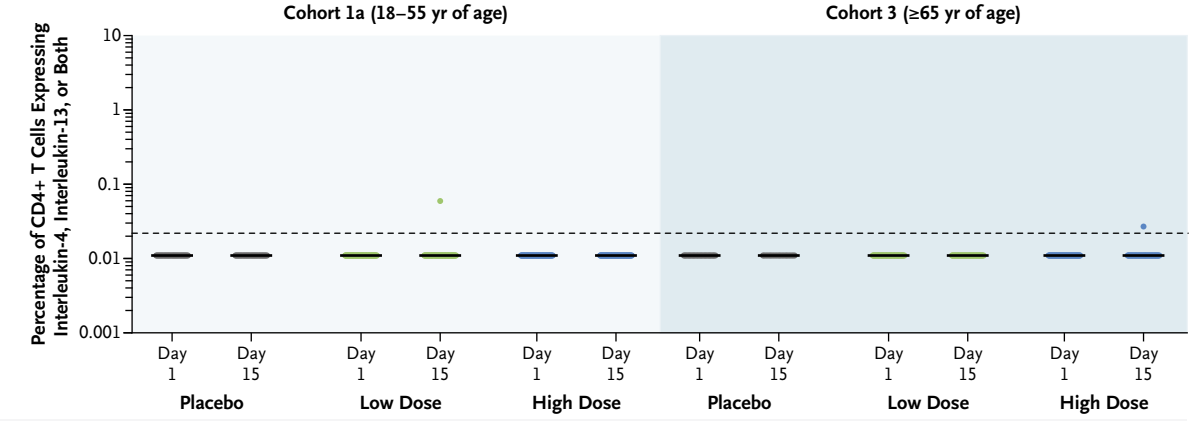
Although all ongoing phase 3 studies of other Covid-19 vaccines have assessed two-dose sched-

A CD4+ Th1 Cells



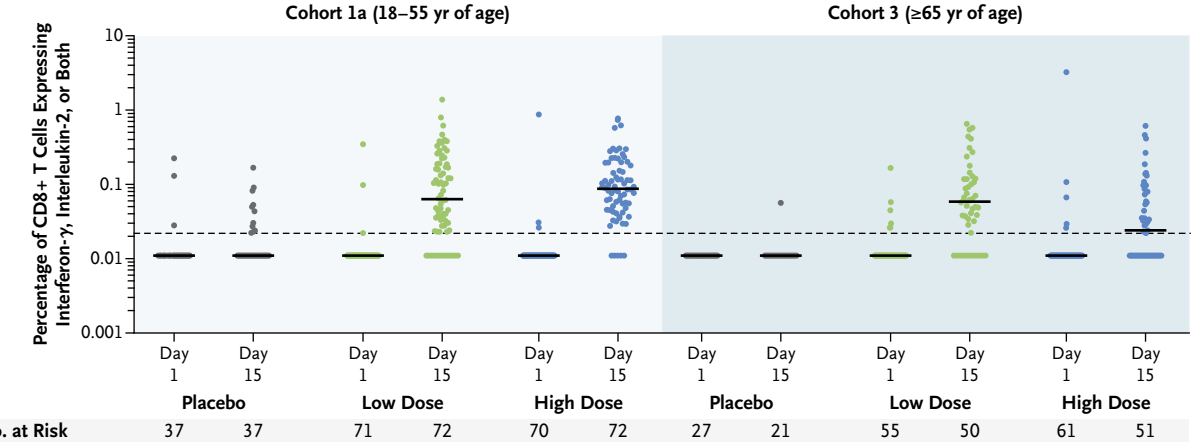
	Placebo		Low Dose		High Dose		Placebo		Low Dose		High Dose	
	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
No. at Risk	37	37	71	72	70	72	30	23	58	53	64	54
Median	<LLOQ	<LLOQ	<LLOQ	0.08	<LLOQ	0.11	<LLOQ	<LLOQ	<LLOQ	0.09	<LLOQ	0.11
Percent Response		0		76		83		0		60		67

B CD4+ Th2 Cells



	Placebo		Low Dose		High Dose		Placebo		Low Dose		High Dose	
	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
No. at Risk	37	37	71	72	70	72	30	23	58	53	64	54
Median	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Percent Response		0		1		0		0		0		2

C CD8+ T Cells



	Placebo		Low Dose		High Dose		Placebo		Low Dose		High Dose	
	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
No. at Risk	37	37	71	72	70	72	27	21	55	50	61	51
Median	<LLOQ	<LLOQ	<LLOQ	0.07	<LLOQ	0.09	<LLOQ	<LLOQ	<LLOQ	0.06	<LLOQ	0.02
Percent Response		0		51		64		0		36		24

Figure 3 (facing page). Cellular Immunogenicity of Ad26.COV2.S.

In CD4+ T cells, the response to low-dose or high-dose vaccine or placebo in type 1 helper T (Th1) cells was characterized by the expression of interferon-γ, interleukin-2, or both, without cytokines expressed by type 2 helper T (Th2) cells (Panel A). The response in CD4+ Th2 cells was characterized by the expression of interleukin-4, interleukin-5, or interleukin-13 (or all three cytokines) plus CD40L (Panel B). In CD8+ T cells, the response was measured by the expression of interferon-γ, interleukin-2, or both (Panel C). In all three panels, the horizontal bars indicate median values on intracellular cytokine staining for individual responses to a SARS-CoV-2 S protein peptide pool in peripheral-blood mononuclear cells at baseline and 15 days after vaccination in a subgroup of participants in cohort 1a (left side) and cohort 3 (right side), according to the receipt of the low or high dose of Ad26.COV2.S or placebo. The horizontal dotted line in each panel indicates the lower limit of quantitation (LLOQ); values below the line have been imputed to half the LLOQ.

ules, a single dose of Ad26.COV2.S elicited a strong humoral response in a majority of vaccine recipients, with the presence of S-binding and neutralizing antibodies in more than 90% of the participants, regardless of either age group or vaccine dose. In addition, during 71 days of follow-up after the first dose, antibody titers further increased and stabilized, which suggests durability of the Ad26.COV2.S-elicited immune response.

The potency of Ad26.COV2.S is supported by the results of our study involving nonhuman primates, in which a single dose provided complete protection against SARS-CoV-2 replication in the lung and near complete protection in the nose.[12] An efficacious single-dose Covid-19 vaccine has obvious logistic advantages over a two-dose vaccine, especially during a pandemic. We observed that among participants between the ages of 18 and 55 years, a second vaccine dose at day 57 further increased the antibody titer, a finding that was also in line with our recent observations in nonhuman primates.[14] Neutralizing-antibody titers against the Ad26 vector that were elicited by the first vaccination did not correlate with the magnitude of response after the second dose. Whether a second dose will provide additional benefit for either improved efficacy or durability in humans, especially in elderly persons in whom the immune response after the first dose tended to be modestly lower than that in younger participants, is currently being studied in a phase 3 clinical trial (ClinicalTrials.gov number, NCT04614948).

The lack of standards and use of different assays complicate the comparison of performance of the various Covid-19 vaccines that are currently in development.[3-6] In addition, comparisons of convalescent serum panels are rather arbitrary, since the reported GMTs have varied according to the composition of the panels (i.e., Covid-19 severity of the donors, time of sampling since disease onset, and other factors).

A theoretical risk of vaccine-associated enhanced respiratory disease (VAERD)[15-17] has been associated with poorly neutralizing humoral immunity and Th2-skewed cellular immune responses. In this trial, all elicited CD4+ T-cell responses to Ad26.COV2.S were Th1-skewed, in line with previous experience with the Ad26-based vaccine platform.[9-11] Data that further minimize the theoretical risk of VAERD are the accompanying consistent CD8+ T-cell responses (albeit occurring at lower levels in older adults than in younger adults) and strong humoral responses.

The demographic characteristics of the participants in our trial confirm the lack of representation of minority groups. This finding is a point of focus in our clinical-development program to ensure the availability of data with respect to groups that seem to be affected most by the Covid-19 pandemic.

Our interim analysis indicates that vaccine candidate Ad26.COV2.S is safe and immunogenic in both younger and older adults. This finding, in combination with the results in preclinical challenge studies,[12,13] has supported our decision to proceed with two phase 3 trials (NCT04505722 and NCT04614948) to evaluate the efficacy of either a single-dose or two-dose regimen of the lower dose (5×10^{10} viral particles) of Ad26.COV2.S.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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APPENDIX

The authors' full names and academic degrees are as follows: Jerald Sadoff, M.D., Mathieu Le Gars, Ph.D., Georgi Shukarev, M.D., Dirk Heerwegh, Ph.D., Carla Truyers, Ph.D., Anne M. de Groot, Ph.D., Jeroen Stoop, Ph.D., Sarah Tete, Ph.D., Wim Van Damme, M.D., Isabel Leroux-Roels, M.D., Pieter-Jan Berghmans, M.D., Murray Kimmel, D.O., Pierre Van Damme, M.D., Jan de Hoon, M.D., William Smith, M.D., Kathryn E. Stephenson, M.D., Stephen C. De Rosa, M.D., Kristen W. Cohen, Ph.D., M. Juliana McElrath, M.D., Emmanuel Cormier, Ph.D., Gert Scheper, Ph.D., Dan H. Barouch, M.D., Jenny Hendriks, Ph.D., Frank Struyf, M.D., Macaya Douoguih, M.D., Johan Van Hoof, M.D., and Hanneke Schuitemaker, Ph.D.

The authors' affiliations are as follows: Janssen Vaccines and Prevention, Leiden, the Netherlands (J. Sadoff, M.L.G., G. Shukarev, A.M.G., J. Stoop, S.T., E.C., G. Scheper, J. Hendriks, M.D., J.V.H., H.S.); Janssen Research and Development, Beerse (D.H., C.T., F.S.), Janssen Clinical Pharmacology Unit, Merksem (W.V.D.), the Center for Vaccinology, Ghent University, Gent (I.L.-R.), SGS Life Sciences (P.-J.B.) and the Center for the Evaluation of Vaccination, University of Antwerp (P.V.D.), Antwerp, and the Center for Clinical Pharmacology, University Hospitals Leuven, Leuven (J. de Hoon) — all in Belgium; Optimal Research, Melbourne, FL (M.K.); the Alliance for Multispecialty Research, Knoxville, TN (W.S.); the Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston (K.E.S., D.H.B.); and the Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle (S.C.D.R., K.W.C., M.J.M.).

REFERENCES

1. World Health Organization. Pneumonia of unknown cause — China. January 5, 2020 (https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/).
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
3. Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 2020;396:467-78.
4. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2 — preliminary report. N Engl J Med 2020;383:1920-31.
5. Zhu F-C, Guan X-H, Li Y-H, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2020;396:479-88.
6. Mulligan MJ, Lyke KE, Kitchin N, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature 2020;586:589-93.
7. Bos R, Rutten L, van der Lubbe JEM, et al. Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses. NPJ Vaccines 2020;5:91.
8. Custers J, Kim D, Leyssen M, et al. Vaccines based on replication incompetent Ad26 viral vectors: standardized template with key considerations for a risk/benefit assessment. Vaccine 2020 October 2 (Epub ahead of print).
9. Anywaine Z, Whitworth H, Kaleebu P, et al. Safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Uganda and Tanzania. J Infect Dis 2019;220:46-56.
10. Williams K, Bastian AR, Feldman RA, et al. Phase 1 safety and immunogenicity study of a respiratory syncytial virus vaccine with an adenovirus 26 vector encoding prefusion F (Ad26.RSV.preF) in adults aged ≥60 years. J Infect Dis 2020;222:979-88.
11. Barouch DH, Tomaka FL, Wegmann F, et al. Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). Lancet 2018;392:232-43.
12. Mercado NB, Zahn R, Wegmann F, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature 2020;586:583-8.
13. Tostanoski LH, Wegmann F, Martinot AJ, et al. Ad26 vaccine protects against SARS-CoV-2 severe clinical disease in hamsters. Nat Med 2020;26:1694-700.
14. Solforosi L, Kuipers H, Rosendal Huber SK, et al. Immunogenicity of one- and two-dose regimens of the Ad26.COV2.S COVID-19 vaccine candidate in adult and aged rhesus macaques. November 17, 2020 (https://www.biorxiv.org/content/10.1101/2020.11.17.368258v1). preprint.
15. Deming D, Sheahan T, Heise M, et al. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. PLoS Med 2006;3(12):e525.
16. Iwata-Yoshikawa N, Uda A, Suzuki T, et al. 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. J Virol 2014;88:8597-614.
17. Yasui F, Kai C, Kitabatake 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. J Immunol 2008;181:6337-48.

Johnson & Johnson Launches Multi-Pronged Response to Coronavirus Global Public Health Threat

Initiating Vaccine Development and Providing Supplies of Antiviral Medicines to China for Investigational Use

NEW BRUNSWICK, N.J., January 29, 2020 – Johnson & Johnson today announced that it is mobilizing resources at its Janssen Pharmaceutical Companies to launch a multi-pronged response to the novel coronavirus (also known as 2019-nCoV or Wuhan coronavirus) outbreak. As part of this work, the Company has initiated efforts to develop a vaccine candidate against 2019-nCoV and broadly collaborate with others to screen a library of antiviral therapies. Identifying compounds with antiviral activity against 2019-nCoV may contribute to providing immediate relief to the current outbreak.

"J&J has a long-standing commitment to fight established and emerging epidemics and is supporting global efforts where we can make the greatest impact. We are collaborating with regulators, healthcare organizations, institutions and communities worldwide to help ensure our research platforms, existing science and outbreak expertise can be maximized to stem this public health threat," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson. "This latest outbreak of a novel pathogen once again reinforces the importance of investing in preparedness, surveillance and response to ensure the world remains ahead of potential pandemic threats."
The vaccine program will leverage Janssen's AdVac® and PER.C6® technologies that provide the ability to rapidly upscale production of the optimal vaccine candidate. These are the same technologies that were used in the development and manufacturing of Janssen's investigational Ebola vaccine, which is currently deployed in the Democratic Republic of the Congo and Rwanda. They were also used to construct the Company's Zika, RSV and HIV vaccine candidates.

Johnson & Johnson's multi-pronged approach also includes a review of known pathways in coronavirus pathophysiology to determine whether previously tested medicines can be used to help patients survive a 2019-nCoV infection and reduce the severity of disease in non-lethal cases. In addition, Janssen has donated 300 boxes of its HIV medication PREZCOBIX® (darunavir/cobicistat) to the Shanghai Public Health Clinical Center and Zhongnan Hospital of Wuhan University for use in research to support efforts in finding a solution against the 2019-nCoV. Furthermore, another 50 boxes have been provided to the Chinese Center for Disease Control and Prevention for laboratory-based investigations (drug-screening for antiviral properties against 2019-nCoV). All shipments have been delivered and, if further donations are required, the Company is open to cooperating with all healthcare institutions and agencies to support efforts in finding a solution against 2019-nCoV.

The requests from the Shanghai Public Health Clinical Center and Zhongnan Hospital of Wuhan University follow a recommendation from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences for investigation of 30 potentially effective compounds, including darunavir – the protease inhibitor component of PREZCOBIX – against 2019-nCoV. Based on anecdotal findings, a protease inhibitor has previously

shown a potential favorable clinical response against severe acute respiratory syndrome (SARS) associated coronavirus.[i]

The Company's expedited research and development schedule is in response to the current 2019-nCoV outbreak in China. The World Health Organization has now confirmed cases of 2019-nCoV across mainland China, with cases also confirmed in countries and territories worldwide, including Australia, Cambodia, Canada, France, Germany, Hong Kong, Japan, Macao, Malaysia, Nepal, Republic of Korea, Singapore, Sri Lanka, Taiwan, Thailand, the U.S.A and Vietnam.[ii],[iii] 2019-nCoV is from a group of viruses called coronaviruses that attack the respiratory system.

PREZCOBIX is a prescription medicine, licensed in many countries including China, for the treatment of human immunodeficiency virus (HIV-1). It has not been proven safe and effective for the treatment of 2019-nCoV and further investigations are required. Cobicistat is from Gilead Sciences, Inc. For specific indications and prescribing information, please consult your local health authorities.

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About Johnson & Johnson
At Johnson & Johnson, we believe good health is the foundation of vibrant lives, thriving communities and forward progress. That's why for more than 130 years, we have aimed to keep people well at every age and every stage of life. Today, as the world's largest and most broadly-based healthcare company, we are committed to using our reach and size for good. We strive to improve access and affordability, create healthier communities, and put a healthy mind, body and environment within reach of everyone, everywhere. We are blending our heart, science and ingenuity to profoundly change the trajectory of health for humanity. Learn more at www.jnj.com. Follow us at @JNJNews.

About the Janssen Pharmaceutical Companies
At Janssen, we're creating a future where disease is a thing of the past. We're the Pharmaceutical Companies of Johnson & Johnson, working tirelessly to make that future a reality for patients everywhere by fighting sickness with science, improving access with ingenuity, and healing hopelessness with heart. We focus on areas of medicine where we can make the biggest difference: Cardiovascular & Metabolism, Immunology, Infectious Diseases & Vaccines, Neuroscience, Oncology, and Pulmonary Hypertension. Learn more at www.janssen.com. Follow us at @JanssenGlobal.

Notice to Investors Concerning Forward-Looking Statements
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changes in behavior and spending patterns of purchasers of health care products and services; changes to applicable laws and regulations, including global health care reforms; and trends toward health care cost containment. A further list and descriptions of these risks, uncertainties and other factors can be found in Johnson & Johnson's Annual Report on Form 10-K for the fiscal year ended December 30, 2018, including in the sections captioned "Cautionary Note Regarding Forward-Looking Statements" and "Item 1A. Risk Factors," and in the company's most recently filed Quarterly Report on Form 10-Q, and the company's subsequent filings with the Securities and Exchange Commission. Copies of these filings are available online at www.sec.gov, www.jnj.com or on request from Johnson & Johnson. None of the Janssen Pharmaceutical Companies nor Johnson & Johnson undertakes to update any forward-looking statement as a result of new information or future events or developments.

[i] Chu CM et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax*. 2004;59:252-256.
[ii] WHO. Novel Coronavirus (2019-nCoV) SITUATION REPORT – 8, 28 JANUARY 2020. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200128-sitrep-8-ncov-cleared.pdf?sfvrsn=8b671ce5_2. Last accessed: January 2020.
[iii] Gov.uk. Wuhan novel coronavirus: epidemiology, virology and clinical features. Available at: https://www.gov.uk/government/publications/wuhan-novel-coronavirus-background-information/wuhan-novel-coronavirus-epidemiology-virology-and-clinical-features. Last accessed: January 2020.

Source: https://www.jnj.com/johnson-johnson-launches-multi-pronged-response-to-coronavirus-global-public-health-threat

Johnson & Johnson Announces Collaboration with U.S. Department of Health & Human Services to Accelerate Development of a Potential Novel Coronavirus Vaccine

Janssen joins forces with Biomedical Advanced Research and Development Authority (BARDA) to protect communities against threat of a global COVID-19 pandemic

NEW BRUNSWICK, N.J., February 11, 2020 – Johnson & Johnson today announced that its Janssen Pharmaceutical Companies will further expedite its investigational coronavirus vaccine program through an expanded collaboration with the Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response (ASPR) at the U.S. Department of Health & Human Services.

The collaborative partnership with BARDA builds on Johnson & Johnson's multipronged response to the new coronavirus disease (COVID-19) outbreak. In addition to Janssen's efforts to develop a vaccine candidate, the Company is working closely with global partners to screen its library of antiviral molecules to accelerate the discovery of potential COVID-19 treatments and provide relief for people in China and around the world.

"Developing an effective vaccine will be critical if we are to protect people against the novel coronavirus and combat future outbreaks," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson. "This partnership will ensure that vital research is made possible at rapid speed and underscores the importance of public-private partnerships to tackle the worldwide novel coronavirus epidemic. We are also in discussions with other partners, that if we have a vaccine candidate with potential, we aim to make it accessible to China and other parts of the world."

Through this agreement, created under an existing U.S. Government's Other Transaction Authority, (HHSO100201700018C), Janssen and BARDA will both contribute to the research and development costs and mobilize resources to rapidly advance the initial stages of Janssen's COVID-19 vaccine development program. BARDA will provide funding to support accelerated development of a vaccine candidate into Phase 1 clinical studies, with options for additional funding to progress a promising candidate. In parallel, Janssen will work to upscale the production and manufacturing capacities required to meet public health needs. Janssen is committed to partnering with multiple stakeholders around the world to address the needs of communities around the world.

"By leveraging long-standing partnerships and proven technology, we can move rapidly to address emerging health threats like this novel coronavirus," said BARDA Director Rick A. Bright, Ph.D. "We are committed to doing everything we can to protect the health of the people in the United States and across the globe."

The vaccine program will leverage Janssen's AdVac® and PER.C6® technologies that provide the ability to rapidly upscale production of the optimal vaccine candidate. These are the same technologies that were used in the development and manufacturing of Janssen's investigational Ebola vaccine, which is currently deployed in the Democratic Republic of the Congo and Rwanda. They were also used to construct the Company's Zika, RSV and HIV vaccine candidates.

COVID-19 belongs to a group of viruses called coronaviruses that attack the respiratory system. There is currently no approved vaccine, treatment or cure for COVID-19.

For more information on Johnson & Johnson's commitment to combatting COVID-19 visit: www.jnj.com/coronavirus.

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Source: https://www.jnj.com/johnson-johnson-announces-collaboration-with-u-s-department-of-health-human-services-to-accelerate-development-of-a-potential-novel-coronavirus-vaccine

Johnson & Johnson Announces Collaboration with the Beth Israel Deaconess Medical Center to Accelerate COVID-19 Vaccine Development

Research well underway as Janssen scientists collaborate with leading virology laboratory led by Dan Barouch, M.D., Ph.D.

NEW BRUNSWICK, N.J., March 13, 2020 – Johnson & Johnson today announced that its Janssen Pharmaceutical Companies have entered a collaboration with the Beth Israel Deaconess Medical Center (BIDMC) to support the development of a preventive vaccine candidate for COVID-19. The parties have commenced preclinical testing of multiple vaccine prospects, with the aim to identify by the end of the month a COVID-19 vaccine candidate for clinical trials.

Janssen is optimistic that, in collaboration with multiple global strategic partners, it can initiate a Phase 1 clinical study of a potential vaccine candidate by the end of the year. In parallel to these efforts, Janssen is preparing to upscale production and manufacturing capacities to levels required to meet global public health vaccination needs.

“It is critical to work with the best scientific minds as we look to rapidly identify and develop solutions to the COVID-19 outbreak,” said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson. “We are grateful for talented and experienced collaboration partners like Dan Barouch and his team at BIDMC. By mobilizing our collective resources, we believe we can leverage the top science and cutting-edge capabilities to respond to this pandemic.”

Janssen’s vaccine program will use the Janssen AdVac® and PER.C6® technologies that provide the ability to rapidly upscale production of an optimal vaccine candidate. The company is leveraging its proven vaccine technology that it is also using to develop its investigational Ebola (which also utilizes its MVA-BN® technology), Zika, RSV and HIV vaccines. Research and collaboration on preclinical work for our Zika and HIV vaccine candidates at the Center for Virology and Vaccine Research at Beth Israel Deaconess Medical Center was foundational to developing these vaccines.

Dan Barouch, M.D., Ph.D., Director of the Center for Virology and Vaccine Research at BIDMC and the Ragon Institute, stated, “We are currently evaluating a series of potential vaccine candidates for COVID-19. This collaboration with Janssen is aimed at the development of a COVID-19 vaccine that would allow for rapid development, large-scale manufacturing, and global delivery.”

Dr. Barouch’s team is well-known for their work on the pathogenesis and immunology of viral infections and the development of vaccine strategies for global infectious diseases.

Johnson & Johnson’s efforts to expedite development and production of a vaccine are enhanced by the existing COVID-19 vaccine collaborations between Janssen and the Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response (ASPR) at the U.S. Department of Health & Human Services.

In addition to Janssen's efforts to develop a vaccine candidate, the Company is working closely with global strategic partners to screen its library of antiviral molecules to accelerate the discovery of potential COVID-19 treatments and provide relief for people around the world.

For more information on Johnson & Johnson's multipronged response to identifying critical solutions to the COVID-19 outbreak, please visit: www.jnj.com/coronavirus.

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Source: https://www.jnj.com/johnson-johnson-announces-collaboration-with-the-beth-israel-deaconess-medical-center-to-accelerate-covid-19-vaccine-development

Johnson & Johnson Announces a Lead Vaccine Candidate for COVID-19; Landmark New Partnership with U.S. Department of Health & Human Services; and Commitment to Supply One Billion Vaccines Worldwide for Emergency Pandemic Use

Johnson & Johnson and BARDA Together Commit More than $1 Billion to Novel Coronavirus Vaccine Research and Development; Company Expects to Initiate Phase 1 Human Clinical Studies of Vaccine Candidate at Latest by September 2020

Johnson & Johnson Will Establish New U.S. Vaccine Manufacturing Capabilities and Additional Production Capacity Outside the U.S. to Begin Production at Risk to Help Ensure Global Vaccine Supply

NEW BRUNSWICK, N.J., March 30, 2020 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced the selection of a lead COVID-19 vaccine candidate from constructs it has been working on since January 2020; the significant expansion of the existing partnership between the Janssen Pharmaceutical Companies of Johnson & Johnson and the Biomedical Advanced Research and Development Authority (BARDA); and the rapid scaling of the Company's manufacturing capacity with the goal of providing global supply of more than one billion doses of a vaccine. The Company expects to initiate human clinical studies of its lead vaccine candidate at the latest by September 2020 and anticipates the first batches of a COVID-19 vaccine could be available for emergency use authorization in early 2021, a substantially accelerated timeframe in comparison to the typical vaccine development process.

Through a landmark new partnership, BARDA, which is part of the Office of the Assistant Secretary for Preparedness and Response (ASPR) at the U.S. Department of Health and Human Services, and Johnson & Johnson together have committed more than $1 billion of investment to co-fund vaccine research, development, and clinical testing. Johnson & Johnson will use its validated vaccine platform and is allocating resources, including personnel and infrastructure globally, as needed, to focus on these efforts. Separately, BARDA and the Company have provided additional funding that will enable expansion of their ongoing work to identify potential antiviral treatments against the novel coronavirus.

As part of its commitment, Johnson & Johnson is also expanding the Company's global manufacturing capacity, including through the establishment of new U.S. vaccine manufacturing capabilities and scaling up capacity in other countries. The additional capacity will assist in the rapid production of a vaccine and will enable the supply of more than one billion doses of a safe and effective vaccine globally. The Company plans to begin production at risk imminently and is committed to bringing an affordable vaccine to the public on a not-for-profit basis for emergency pandemic use.

Alex Gorsky, Chairman and Chief Executive Officer, Johnson & Johnson, said, “The world is facing an urgent public health crisis and we are committed to doing our part to make a COVID-19 vaccine available and affordable globally as quickly as possible. As the world’s largest healthcare company, we feel a deep responsibility to improve the health of people around the world every day. Johnson & Johnson is well positioned through our combination of scientific expertise, operational scale and financial strength to bring our resources in collaboration with others to accelerate the fight against this pandemic.”

Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson, said, “We greatly value the U.S. government’s confidence and support for our R&D efforts. Johnson & Johnson’s global team of experts has ramped up our research and development processes to unprecedented levels, and our teams are working tirelessly alongside BARDA, scientific partners, and global health authorities. We are very pleased to have identified a lead vaccine candidate from the constructs we have been working on since January. We are moving on an accelerated timeline toward Phase 1 human clinical trials at the latest by September 2020 and, supported by the global production capability that we are scaling up in parallel to this testing, we expect a vaccine could be ready for emergency use in early 2021.”

Johnson & Johnson’s Lead COVID-19 Vaccine Candidate
Johnson & Johnson began efforts in January 2020, as soon as the novel coronavirus (COVID-19) sequence became available, to research potential vaccine candidates. Research teams at Janssen, in collaboration with Beth Israel Deaconess Medical Center, part of Harvard Medical School, constructed and tested multiple vaccine candidates using the Janssen AdVac® technology.

Through collaborations with scientists at multiple academic institutions, the vaccine constructs were then tested to identify those with the most promise in producing an immune response in preclinical testing.

Based on this work, Johnson & Johnson has identified a lead COVID-19 vaccine candidate (with two back-ups), which will progress into the first manufacturing steps. Under an accelerated timeline, the Company is aiming to initiate a Phase 1 clinical study in September 2020, with clinical data on safety and efficacy expected to be available by the end of the year. This could allow vaccine availability for emergency use in early 2021. For comparison, the typical vaccine development process involves a number of different research stages, spanning 5 to 7 years, before a candidate is even considered for approval.

For more than 20 years, Johnson & Johnson has invested billions of dollars in antivirals and vaccine capabilities. The COVID-19 vaccine program is leveraging Janssen’s proven AdVac® and PER.C6® technologies that provide the ability to rapidly develop new vaccine candidates and upscale production of the optimal vaccine candidate. The same technology was used to develop and manufacture the Company’s Ebola vaccine and construct our Zika, RSV, and HIV vaccine candidates which are in Phase 2 or Phase 3 clinical development stages.

Expanded Antiviral Research
In addition to the vaccine development efforts, BARDA and Johnson & Johnson have also expanded their partnership to accelerate Janssen’s ongoing work in screening compound libraries, including compounds from other pharmaceutical companies. The

Company's aim is to identify potential treatments against the novel coronavirus. Johnson & Johnson and BARDA are both providing funding as part of this partnership. These antiviral screening efforts are being conducted in partnership with the Rega Institute for Medical Research (KU Leuven/University of Leuven), in Belgium.

As announced in February 2020, the Company and BARDA have been working closely with global partners to screen Janssen's library of antiviral molecules to accelerate the discovery of potential COVID-19 treatments.

COVID-19 belongs to a group of viruses called coronaviruses that attack the respiratory system. There is currently no approved vaccine, treatment or cure for COVID-19.

For more information on Johnson & Johnson's multi-pronged approach to combatting the pandemic, visit: www.jnj.com/coronavirus.

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Johnson & Johnson Announces Collaboration to Expand Manufacturing Capabilities For its COVID-19 Vaccine Candidate in Support of the Company's Goal to Supply More Than One Billion Vaccine Doses Globally

Company Signs Agreement with Emergent BioSolutions in the U.S. As Part of its Investment

First in Series of Anticipated Strategic Collaborations Designed to Further the Company's Goal of Ensuring Global Supply of a Safe and Effective Vaccine for COVID-19

NEW BRUNSWICK, N.J., April 23, 2020 - Johnson & Johnson (the Company) (NYSE: JNJ) today announced a collaboration between the Janssen Pharmaceutical Companies of Johnson & Johnson and Emergent BioSolutions, Inc. to support the manufacturing of its lead investigational COVID-19 vaccine candidate. This is the first in a series of prospective global collaboration agreements designed to accelerate manufacturing of Johnson & Johnson's COVID-19 vaccine candidate, and further the Company's goal to supply more than one billion doses of the vaccine globally.

Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson, said, "We have set a high bar. Johnson & Johnson has committed to rapidly produce and supply more than one billion doses of a safe and effective vaccine globally. Our collaboration with Emergent is proof that we are moving quickly to deliver on that promise."

Under the terms of this manufacturing agreement, Johnson & Johnson is investing to expand drug substance capacity related to the vaccine candidate. Emergent will provide drug substance manufacturing services with its molecule-to-market CDMO offering, beginning in 2020, and will also reserve operations capacity to potentially support commercial manufacturing of Johnson & Johnson's COVID-19 vaccine candidate leveraging Janssen's proven AdVac® and PER.C6® technologies beginning in 2021.

The Company has already begun preparations for clinical vaccine production at its facility in Leiden, the Netherlands, with the aim of initiating Phase 1 human clinical studies of its vaccine candidate in September 2020. Johnson & Johnson will begin production at risk and is committed to bringing an affordable vaccine to the public on a not-for-profit basis for emergency pandemic use.

Simultaneously, Johnson & Johnson is also aiming to rapidly scale up vaccine manufacturing capabilities globally, including increasing capacity in countries outside the U.S. The additional global capacity will assist in the rapid production of a vaccine and enable the supply of more than one billion doses of a safe and effective vaccine to people around the world.

For more than 20 years, Johnson & Johnson has invested billions of dollars in antivirals and vaccine capabilities. The COVID-19 vaccine program leverages Janssen's

proven AdVac® and PER.C6® technologies that provide the ability to rapidly develop new vaccine candidates and upscale production of the optimal vaccine candidate. The same technology was used to develop and manufacture the Company's investigational Ebola vaccine and construct our RSV and HIV vaccine candidates which are in Phase 2 or Phase 3 clinical development stages.

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Johnson & Johnson Announces Acceleration of its COVID-19 Vaccine Candidate; Phase 1/2a Clinical Trial to Begin in Second Half of July

Johnson & Johnson Continues to Build its Manufacturing Capacity and Partnerships to Meet Commitment and Global Need

NEW BRUNSWICK, N.J., June 10, 2020 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced that through its Janssen Pharmaceutical Companies (Janssen) it has accelerated the initiation of the Phase 1/2a first-in-human clinical trial of its investigational SARS-CoV-2 vaccine, Ad26.COV2-S, recombinant. Initially scheduled to begin in September, the trial is now expected to commence in the second half of July.

Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson, said, “Based on the strength of the preclinical data we have seen so far and interactions with the regulatory authorities, we have been able to further accelerate the clinical development of our investigational SARS-CoV-2 vaccine, Ad26.COV2-S, recombinant. Simultaneously, we are continuing our efforts to build important global partnerships and invest in our vaccine production technology and manufacturing capabilities. Our goal is to ensure we can deliver a vaccine to the world and protect people everywhere from this pandemic.”

The randomized, double-blind, placebo-controlled Phase 1/2a study will evaluate the safety, reactogenicity (response to vaccination), and immunogenicity (immune response) of the investigational SARS-CoV-2 vaccine, Ad26.COV2-S, recombinant in 1045 healthy adults aged 18 to 55 years, as well as adults aged 65 years and older. The study will take place in the U.S. and Belgium.

The Company is in discussions with the National Institutes of Allergy and Infectious Diseases with the objective to start the Phase 3 SARS-CoV-2 vaccine, Ad26.COV2-S, recombinant, clinical trial ahead of its original schedule, pending outcome of Phase 1 studies and approval of regulators.

As the Company progresses the clinical development of its investigational SARS-CoV-2 vaccine, Ad26.COV2-S, recombinant, it continues to increase manufacturing capacity and is in active discussions with global partners to ensure worldwide access. The Company committed to the goal of supplying more than one billion doses globally through the course of 2021, provided the vaccine is safe and effective.

Johnson & Johnson’s efforts to expedite development and production of a SARS-CoV-2 vaccine are enhanced by a collaboration between Janssen and the Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response (ASPR) at the U.S. Department of Health & Human Services.

COVID-19 is caused by SARS-CoV-2, which belongs to a group of viruses called coronaviruses that attack the respiratory system. There is currently no approved vaccine for COVID-19.

For more information on Johnson & Johnson's multi-pronged approach to combatting the pandemic, visit: www.jnj.com/coronavirus.

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Source: https://www.jnj.com/johnson-johnson-announces-acceleration-of-its-covid-19-vaccine-candidate-phase-1-2a-clinical-trial-to-begin-in-second-half-of-july

Single Dose of Johnson & Johnson COVID-19 Vaccine Candidate Demonstrates Robust Protection in Pre-clinical Studies

Study published in Nature *shows J&J's investigational SARS-CoV-2 vaccine elicits a strong immune response that protects against subsequent infection*

First-in-human Phase 1/2a clinical trial now underway in United States and Belgium; Phase 3 clinical trial expected to commence in September

NEW BRUNSWICK, N.J., July 30, 2020 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced that its lead vaccine candidate protected against infection with SARS-CoV-2, the virus that causes COVID-19, in pre-clinical studies. The data, published in *Nature*, show the Company's investigational adenovirus serotype 26 (Ad26) vector-based vaccine elicited a robust immune response as demonstrated by "neutralizing antibodies," successfully preventing subsequent infection and providing complete or near-complete protection in the lungs from the virus in non-human primates (NHPs) in the pre-clinical study. Based on the strength of the data, a Phase 1/2a first-in-human clinical trial of the vaccine candidate, Ad26.COV2.S, in healthy volunteers, has now commenced in the United States and Belgium.

"We are excited to see these pre-clinical data because they show our SARS-CoV-2 vaccine candidate generated a strong antibody response and provided protection with a single dose. The findings give us confidence as we progress our vaccine development and upscale manufacturing in parallel, having initiated a Phase 1/2a trial in July with the intention to move into a Phase 3 trial in September," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson.

The robust Janssen COVID-19 clinical trial program, including the Phase 1/2a clinical trial and the Phase 3 clinical trial program, will evaluate both one- and two-dose regimens of Ad26.COV2.S in parallel studies. The Phase 1/2a trial will evaluate the safety, reactogenicity (expected reactions to vaccination, such as swelling or soreness), and immunogenicity of Ad26.COV2.S in over 1,000 healthy adults aged 18 to 55 years, as well as adults aged 65 years and older. Planning also is underway for a Phase 2a study in the Netherlands, Spain and Germany and a Phase 1 study in Japan. For more information about these studies, please visit www.clinicaltrials.gov.

As the Company plans its COVID-19 Phase 3 clinical development program, discussions are underway with partners with the objective to start a pivotal Phase 3 clinical trial of the single vaccine dose versus placebo in September, pending the interim data of the Phase 1 and 2 trials and approval of regulators. Simultaneously, the Company also is planning to start a parallel Phase 3 clinical trial of a two-dose regimen versus placebo.

The Company also will emphasize representation of populations that have been disproportionately impacted by the pandemic as it designs and implements its COVID-19 Phase 3 trial program. In the United States, this would include significant representation of Blacks, Hispanic/Latinx and participants over 65 years of age.

The pre-clinical studies were conducted by researchers from Beth Israel Deaconess Medical Center (BIDMC) in collaboration with the Janssen Pharmaceutical Companies of Johnson & Johnson and others as part of its ongoing collaboration to accelerate the development of a SARS-CoV-2 vaccine.

Dan Barouch, M.D., Ph.D., Director of the Center for Virology and Vaccine Research at BIDMC and the Ragon Institute, stated, “The pre-clinical data, generated in collaboration with the Johnson & Johnson team, highlights the potential of this SARS-CoV-2 vaccine candidate. Moreover, the data suggest that antibody levels may serve as a biomarker for vaccine-mediated protection.”

In the studies, researchers first immunized the NHPs with a panel of vaccine prototypes, and then challenged them with SARS-CoV-2 infection. The scientists found that, of seven vaccine prototypes tested in the study, Ad26.COV2.S (referred to in the *Nature* article as Ad26-S.PP), elicited the highest levels of neutralizing antibodies to SARS-CoV-2. The level of antibodies correlated with the level of protection, confirming previous observations and suggesting they could be a potential biomarker for vaccine-mediated protection. The six NHPs that received a single immunization with Ad26.COV2.S showed no detectable virus in the lower respiratory tract after exposure to SARS-CoV-2, and only one of six showed very low levels of the virus in a nasal swab at two time points.

“As we collectively battle this pandemic, we remain deeply committed to our goal of providing a safe and effective vaccine to the world. Our pre-clinical results give us reason to be optimistic as we initiate our first-in-human clinical trial, and we are excited to enter the next stage in our research and development toward a COVID-19 vaccine. We know that, if successful, this vaccine can be rapidly developed, produced on a large scale and delivered around the world,” said Mathai Mammen, M.D., Ph.D., Global Head, Janssen Research & Development, LLC, Johnson & Johnson.

The Company’s fundamental responsibility is to provide patients, consumers and healthcare providers with products that are as safe and effective as possible. Johnson & Johnson takes an evidence- and science-based, ethics- and values-driven approach to medical safety, putting patient and consumer wellbeing first and foremost in its decision making and actions, with an emphasis on transparency.

As Johnson & Johnson progresses the clinical development of SARS-CoV-2, the Company continues to increase manufacturing capacity and is in active discussions with global strategic partners to support worldwide access. Johnson & Johnson aims to meet its goal to supply more than one billion doses globally through the course of 2021, provided the vaccine is safe and effective.

This project has been funded in whole or in part with Federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, under Other Transaction Agreement HHSO100201700018C.

For more information on Johnson & Johnson’s multi-pronged approach to combatting the pandemic, visit: www.jnj.com/coronavirus.

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About Johnson & Johnson
At Johnson & Johnson, we believe good health is the foundation of vibrant lives, thriving communities and forward progress. That's why for more than 130 years, we have aimed to keep people well at every age and every stage of life. Today, as the world's largest and most broadly-based healthcare company, we are committed to using our reach and size for good. We strive to improve access and affordability, create healthier communities, and put a healthy mind, body and environment within reach of everyone, everywhere. We are blending our heart, science and ingenuity to profoundly change the trajectory of health for humanity. Learn more at www.jnj.com. Follow us at @JNJNews.

About the Janssen Pharmaceutical Companies
At Janssen, we're creating a future where disease is a thing of the past. We're the Pharmaceutical Companies of Johnson & Johnson, working tirelessly to make that future a reality for patients everywhere by fighting sickness with science, improving access with ingenuity, and healing hopelessness with heart. We focus on areas of medicine where we can make the biggest difference: Cardiovascular & Metabolism, Immunology, Infectious Diseases & Vaccines, Neuroscience, Oncology, and Pulmonary Hypertension. Learn more at www.janssen.com. Follow us at @JanssenGlobal.

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Source: https://www.jnj.com/single-dose-of-johnson-johnson-covid-19-vaccine-candidate-demonstrates-robust-protection-in-pre-clinical-studies

Johnson & Johnson Announces Collaboration in Principle with the United Kingdom on Additional Phase 3 Study and Agreement to Supply its COVID-19 Vaccine Candidate

Company working to ensure broad global access to COVID-19 vaccine candidate, following approval from regulators

August 14, 2020. Janssen Pharmaceutica NV, one of the Janssen Pharmaceutical Companies of Johnson & Johnson (NYSE: JNJ) (the Company), has agreed in principle to collaborate with the United Kingdom of Great Britain and Northern Ireland (the UK Government) on a global Phase 3 clinical trial to explore the two-dose regimen of Janssen's SARS-CoV-2 vaccine candidate, Ad26.COV2.S. This global study will run in parallel to the Phase 3 trial investigating the single-dose regimen of Ad26.COV2.S.

In addition, Janssen has agreed in principle to supply the UK Government with doses of its SARS-CoV-2 vaccine candidate, Ad26.COV2.S. The availability of the vaccine candidate is subject to its successful development and regulatory approval.

"We are delighted to work with the UK Government on the global Phase 3 clinical programme for our COVID-19 vaccine candidate, and to ensure it is made available to citizens around the world, if proven to be effective with a good safety profile. Ending the current COVID-19 pandemic will take a global effort, and this agreement is an important example of how we can begin to address this significant challenge through collaborative research," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson.

The parties will negotiate a final advance purchase agreement in due course under which the UK Government would initially purchase 30 million doses of Janssen's Ad26.COV2.S vaccine candidate on a not-for-profit basis for emergency pandemic use. The agreement will also provide the option for an additional purchase by the UK Government of up to a further 22 million vaccine doses.

The Phase 1/2a first-in-human clinical trial of the vaccine candidate, Ad26.COV2.S, is underway in healthy volunteers in the United States and Belgium. Planning is underway for the Phase 3 programme which is subject to interim data of the Phase 1 trials and approval of regulators.

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Source:
https://www.janssen.com/emea/sites/www_janssen_com_emea/files/johnson_johnson_announces_collaboration_in_principle_with_the_united_kingdom_on_additional_phase_3_study_and_agreement_to_supply_its_covid-19_vaccine_candidate_.pdf

Johnson & Johnson Announces that Janssen's COVID-19 Investigational Vaccine Candidate Prevents Severe Clinical Disease in Pre-clinical Studies

September 03, 2020. Janssen's lead SARS-CoV-2 investigational vaccine candidate, Ad26.COV2.S, prevented severe clinical disease in Syrian golden hamsters, upon challenge with SARS-CoV-2, the virus that causes COVID-19 in people. The data, published today in *Nature Medicine*, demonstrated that the Company's investigational adenovirus serotype 26 (Ad26) vector-based vaccine elicited an immune response as demonstrated by "neutralizing antibodies" and prevented severe clinical disease – including weight loss, pneumonia and mortality – in Syrian golden hamsters upon challenge.

This publication follows Johnson & Johnson's recent announcement that its vaccine candidate elicited an immune response in a pre-clinical study in non-human primates (NHP), that correlated with protection against SARS-CoV-2, providing complete protection against viral replication in the lungs. The latest research tested the vaccine candidate in Syrian golden hamsters, as they are more susceptible to clinical disease than NHPs, which typically do not get severe disease.

"This pre-clinical study further validates our confidence in our SARS-CoV-2 vaccine candidate," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson. "With our Phase 3 trials planned to start this month, we remain committed to expanding our manufacturing and distribution capabilities to enable global access to our SARS-CoV-2 vaccine candidate should it prove to be safe and effective in humans."

The pre-clinical studies were conducted by researchers from Beth Israel Deaconess Medical Center (BIDMC) in collaboration with the Janssen Pharmaceutical Companies of Johnson & Johnson and others as part of its ongoing collaboration to accelerate the development of a SARS-CoV-2 vaccine.

In the pre-clinical study, researchers first immunized Syrian golden hamsters with a single injection of the Ad26-based SARS-CoV-2 vaccine, which induced neutralizing antibodies in all the vaccinated animals. Four weeks later, the animals were exposed to a high dose of SARS-CoV-2 virus. These vaccinated animals lost less weight and had less virus in their lungs and other organs than unvaccinated control animals. Mortalities were absent in vaccinated animals. Moreover, the researchers found that neutralizing antibody responses were inversely correlated with weight loss and viral replication in the lung.

Ad26.COV2.S is currently being evaluated in clinical studies to establish the performance of the vaccine candidate in humans.

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Johnson & Johnson Initiates Pivotal Global Phase 3 Clinical Trial of Janssen's COVID-19 Vaccine Candidate

First participants dosed in Phase 3 trial (ENSEMBLE) evaluating safety and efficacy of Janssen's COVID-19 vaccine candidate, JNJ-78436735, also known as Ad26.COV2.S

NEW BRUNSWICK, N.J., September 23, 2020 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced the launch of its large-scale, pivotal, multi-country Phase 3 trial (ENSEMBLE) for its COVID-19 vaccine candidate, JNJ-78436735, being developed by its Janssen Pharmaceutical Companies. The initiation of the ENSEMBLE trial follows positive interim results from the Company's Phase 1/2a clinical study, which demonstrated that the safety profile and immunogenicity after a single vaccination were supportive of further development. These results have been submitted to medRxiv and are due to be published online imminently. Based on these results and following discussions with the U.S. Food and Drug Administration (FDA), ENSEMBLE will enroll up to 60,000 volunteers across three continents and will study the safety and efficacy of a single vaccine dose versus placebo in preventing COVID-19.

Johnson & Johnson has continued the scaling up of its manufacturing capacity and remains on track to meet its goal of providing one billion doses of a vaccine each year. The Company is committed to bringing an affordable vaccine to the public on a not-for-profit basis for emergency pandemic use and anticipates the first batches of a COVID-19 vaccine to be available for emergency use authorization in early 2021, if proven to be safe and effective.

Johnson & Johnson will develop and test its COVID-19 vaccine candidate in accordance with high ethical standards and sound scientific principles. The Company is committed to transparency and sharing information related to the Phase 3 ENSEMBLE study – including the study protocol.

"As COVID-19 continues to impact the daily lives of people around the world, our goal remains the same – leveraging the global reach and scientific innovation of our company to help bring an end to this pandemic," said Alex Gorsky, Chairman and Chief Executive Officer, Johnson & Johnson. "As the world's largest healthcare company, we are bringing to bear our best scientific minds, and rigorous standards of safety, in collaboration with regulators, to accelerate the fight against this pandemic. This pivotal milestone demonstrates our focused efforts toward a COVID-19 vaccine that are built on collaboration and deep commitment to a robust scientific process. We are committed to clinical trial transparency and to sharing information related to our study, including details of our study protocol."

"We remain fully focused on developing an urgently needed, safe and effective COVID-19 vaccine for people around the world," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson. "We greatly value the collaboration and support from our scientific partners and global health authorities as our global team of experts work tirelessly on the development of the vaccine and scaling up our production capacity with a goal to deliver a vaccine for emergency use authorization in early 2021."

The Janssen COVID-19 vaccine candidate leverages the Company's AdVac® technology platform, which was also used to develop and manufacture Janssen's European Commission approved Ebola vaccine and construct its Zika, RSV, and HIV vaccine candidates. Janssen's AdVac® technology platform has been used to vaccinate more than 100,000 people to date across Janssen's investigational vaccine programs.

With Janssen's AdVac® technology, the vaccine, if successful, is estimated at launch to remain stable for two years at -20 °C and at least three months at 2-8° C. This makes the vaccine candidate compatible with standard vaccine distribution channels and would not require new infrastructure to get it to the people who need it.

PHASE 3 ENSEMBLE STUDY

The Phase 3 ENSEMBLE study is a randomized, double-blind, placebo-controlled clinical trial designed to evaluate the safety and efficacy of a single vaccine dose versus placebo in up to 60,000 adults 18 years old and older, including significant representation from those that are over age 60. The trial will include those both with and without comorbidities associated with an increased risk for progression to severe COVID-19, and will aim to enroll participants in Argentina, Brazil, Chile, Colombia, Mexico, Peru, South Africa and the United States. In order to evaluate the effectiveness of Janssen's COVID-19 vaccine, countries and clinical trial sites which have a high incidence of COVID-19 and the ability to achieve a rapid initiation will be activated.

Built on a legacy of purpose-driven actions and a commitment to diversity and inclusion, the Company aims to achieve representation of populations that have been disproportionately impacted by the pandemic in the implementation of its COVID-19 Phase 3 trial program. In the U.S., this includes significant representation of Black, Hispanic/Latinx, American Indian and Alaskan Native participants.

ENSEMBLE is being initiated in collaboration with the Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response at the U.S. Department of Health and Human Services (HHS) under Other Transaction Agreement HHSO100201700018C, and the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) at HHS.

In parallel, the Company has also agreed in principle to collaborate with the United Kingdom of Great Britain and Northern Ireland (the UK Government) on a separate Phase 3 clinical trial in multiple countries to explore a two-dose regimen of Janssen's vaccine candidate.

"With our vaccine candidate now in our global Phase 3 trial, we are one step closer to finding a solution for COVID-19. We used a highly scientific and evidence-based approach to select this vaccine candidate. We are extremely grateful for the tireless efforts of our researchers and for the vital contributions of those participants who have volunteered to take part in our studies. Together, we are working to help combat this pandemic," said Mathai Mammen, M.D., Ph.D., Global Head, Janssen Research & Development, LLC, Johnson & Johnson.

The Company is in ongoing discussions with many stakeholders, including national governments and global organizations, as part of its efforts to meet its commitment to

make the vaccine candidate accessible globally, provided the vaccine is demonstrated to be safe and effective and following regulatory approval.

For more information on Johnson & Johnson's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

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Source: https://www.jnj.com/johnson-johnson-initiates-pivotal-global-phase-3-clinical-trial-of-janssens-covid-19-vaccine-candidate

Johnson & Johnson Posts Interim Results from Phase 1/2a Clinical Trial of its Janssen COVID-19 Vaccine Candidate

Interim analysis from Phase 1/2a First-in-Human trial supports further clinical development of investigational vaccine candidate JNJ-78436735 (also known as Ad26.COV2.S)

October 4, 2020 Interim analysis from the ongoing Phase 1/2a clinical trial of the Janssen COVID-19 vaccine candidate (JNJ-78436735) was posted today on the pre-print server *medRxiv*.

The data demonstrate that a single dose of JNJ-78436735 induced a strong neutralizing antibody response in nearly all participants aged 18 years and older and was generally well-tolerated. Immune responses were similar across the age groups studied, including older adults.

The ongoing Phase 1/2a clinical trial is designed to study the safety and immunogenicity of two dose levels of the Janssen COVID-19 vaccine, and as single and two-dose schedules. The interim analysis showed that a single dose induced a robust immune response and was generally well-tolerated. These data are consistent with preclinical studies, published in the scientific journal *Nature*, which showed that a single dose of the vaccine successfully prevented subsequent infection and provided complete protection in the lungs of nonhuman primates.

Based on these findings, the single dose of the Janssen COVID-19 vaccine candidate of $5x10^{10}$ virus particles (vp) has been selected for further evaluation in the Phase 3 ENSEMBLE clinical trial. The Company also plans on running a Phase 3 clinical trial of a two-dose regimen of JNJ-78436735 versus placebo later this year.

The full set of results will be published once the complete Phase 1/2a trial data are available.

Immune Response Data
Seroconversion (the development of detectable antibodies) was observed in 99 percent of participants aged 18-55 years of age. 98 percent of participants were positive for neutralizing antibodies against SARS-CoV-2 at day 29 post-vaccination. The Janssen COVID-19 vaccine candidate elicited strong antibody responses, strong T cell responses, and a Th1 response, believed to be protective against the risk of vaccine-associated enhanced respiratory disease.

Immunogenicity (the ability to trigger an immune response) data from participants aged 65 years of age and above were available for the first 15 participants at the time of this post, with strong humoral and cellular immune responses elicited in all elderly participants who received a single dose of Janssen's COVID-19 vaccine candidate.

Immune responses were similar across the age groups studied, including older adults.

Safety and Tolerability Data
Interim safety data from the Phase 1/2a trial indicated that the majority of adverse events reported were mild to moderate (grade 1 and grade 2) in severity and generally occurred on the day of vaccination with symptoms generally resolving that day, or the following day. Two serious adverse events were reported, the first for hypotension which the investigator determined to not be vaccine related, and the second was a participant with a fever who was hospitalized due to suspicion of having COVID-19 but recovered within 12 hours. No grade 4 (life-threatening) adverse events, solicited or unsolicited, were reported in any cohort, and no participant discontinued the study due to an adverse event. The analysis showed there was a trend toward higher reactogenicity with the higher vaccine dose and with younger age.

In clinical studies investigating vaccines, it is well known that vaccines often induce local and systemic side-effects that are mild, to moderate, and transient without consequences. In vaccine clinical trials these type of side effects are actively sought ("solicited"). The interim safety data in this Phase 1/2a study is blinded to ensure participants and trial investigators are not made aware which participants received a single dose of Janssen's COVID-19 vaccine candidate versus a placebo.

Study Design
This Phase 1/2a multi-center, randomized, double-blind, placebo-controlled trial aims to evaluate the safety, reactogenicity, and immunogenicity of Janssen's COVID-19 vaccine candidate at two dose levels, administered intramuscularly as single-dose or two-dose schedules, eight weeks apart, in healthy adults 18-55 and greater than 65 years of age. The study is ongoing at multiple clinical sites in Belgium and the United States.

For more information on Johnson & Johnson's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

Additional Information
"We are very encouraged by the immunogenicity of our COVID-19 vaccine candidate based on the antibody and T-cell data that was seen after a single dose and reported in the interim analysis of our Phase 1/2a trial," said Mathai Mammen, M.D., Ph.D., Global Head of Janssen Research & Development, Johnson & Johnson. "Our scientific confidence of strong vaccine efficacy is based on mathematical modelling that relates the antibody levels we have seen in humans to the levels required for disease protection that we observed in non-human primates. We are now evaluating a single-dose of our COVID-19 vaccine candidate versus placebo in the ENSEMBLE Phase 3 study."

At day 29, 97 percent of participants had detectable wild type SARS-CoV-2 virus neutralizing antibodies, with a titer higher than 1:100 in >80% of participants.

A single dose of a safe and effective vaccine would offer a significant advantage during a global pandemic emergency. However, a two-dose schedule *may* have potential to offer enhanced durability in some participants. Therefore, Janssen is studying a single-dose of its vaccine candidate in its pivotal ENSEMBLE trial and plans to run a Phase 3 clinical trial with a two-dose regimen of JNJ-78436735 versus placebo later this year.

The Phase 1/2a interim analyses also included data from one set of human convalescent serum (HCS) samples and noted that the geometric mean titers (GMT) in serum of vaccine recipients were lower than the mean titers in this HCS panel. Panels

used to assess the immunogenicity of vaccine candidates are not standardized and therefore cannot be directly compared.

As compared to the HCS panel used by the Company to validate the NHP immunogenicity studies, the GMT of neutralizing antibodies induced by a single dose of our vaccine in this study was substantially higher.

In a separate group of participants vaccinated in the Janssen Phase 1/2a COVID-19 vaccine study (data to be published), an alternative human convalescent panel was used. In this panel we have observed the antibody titers induced by our vaccine were higher than this panel.

The Janssen Ad26 vaccine platform (known as AdVac®) has substantial clinical experience and has been used to vaccinate over 100,000 people across its global clinical programs.

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Source: https://www.jnj.com/johnson-johnson-posts-interim-results-from-phase-1-2a-clinical-trial-of-its-janssen-covid-19-vaccine-candidate

Johnson & Johnson Temporarily Pauses All Dosing in Our Janssen COVID-19 Vaccine Candidate Clinical Trials

United States – October 13, 2020. At Johnson & Johnson, there is no greater priority than the safety and well being of the people we serve every day around the world. We are committed to providing transparent updates throughout the clinical development process of our vaccine candidate, in compliance with regulatory standards and our own high ethical and scientific principles.

We have temporarily paused further dosing in all our COVID-19 vaccine candidate clinical trials, including the Phase 3 ENSEMBLE trial, due to an unexplained illness in a study participant. Following our guidelines, the participant's illness is being reviewed and evaluated by the ENSEMBLE independent Data Safety Monitoring Board (DSMB) as well as our internal clinical and safety physicians.

Adverse events – illnesses, accidents, etc. - even those that are serious, are an expected part of any clinical study, especially large studies. Based on our strong commitment to safety, all clinical studies conducted by the Janssen Pharmaceutical Companies of Johnson & Johnson have prespecified guidelines. These ensure our studies may be paused if an unexpected serious adverse event (SAE) that might be related to a vaccine or study drug is reported, so there can be a careful review of all of the medical information before deciding whether to restart the study.

We must respect this participant's privacy. We're also learning more about this participant's illness, and it's important to have all the facts before we share additional information.

SAEs are not uncommon in clinical trials, and the number of SAEs can reasonably be expected to increase in trials involving large numbers of participants. Further, as many trials are placebo-controlled, it is not always immediately apparent whether a participant received a study treatment or a placebo.

"Study Pause" vs. "Regulatory Hold:" What's the Difference?

While these terms are sometimes used interchangeably, there is a significant distinction between a study pause and a regulatory hold of a clinical trial.

A study pause, in which recruitment or dosing is paused by the study sponsor, is a standard component of a clinical trial protocol. As noted in the ENSEMBLE study protocol, Johnson & Johnson has robust mechanisms in place to protect the safety of participants in its clinical trials. While the Company informs all study investigators, we typically do not communicate study pauses publicly.

A regulatory hold of a clinical trial is a requirement by a regulatory health authority, such as the U.S. Food and Drug Administration (FDA). As outlined in our transparency commitments, we proactively disclose any regulatory hold of a pivotal clinical trial.

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Source: https://www.janssen.com/johnson-johnson-temporarily-pauses-all-dosing-our-janssen-covid-19-vaccine-candidate-clinical-trials

Johnson & Johnson Prepares to Resume Phase 3 ENSEMBLE Trial of its Janssen COVID-19 Vaccine Candidate in the U.S.

Updated Statement October 23, 2020. Johnson & Johnson announced today that it is preparing to resume recruitment in the pivotal Phase 3 ENSEMBLE trial of its investigational Janssen COVID-19 vaccine in the United States after a temporary pause.

The independent Data Safety and Monitoring Board (DSMB) overseeing the ENSEMBLE study has recommended resuming trial recruitment. Following consultation with the U.S. Food and Drug Administration (FDA), preparations to resume the trial in the United States, including submissions for approval by the Institutional Review Boards, are now underway. Discussions with other regulators around the world to resume the clinical trial program are progressing.

After a thorough evaluation of a serious medical event experienced by one study participant, no clear cause has been identified. There are many possible factors that could have caused the event. Based on the information gathered to date and the input of independent experts, the Company has found no evidence that the vaccine candidate caused the event.

At Johnson & Johnson, there is no greater priority than the health and safety of the people we serve every day around the world. Our primary goal is to ensure the safety, well-being and privacy of the participants and all those involved in our trials.

Janssen is committed to respecting study participant privacy and the integrity of the clinical trial in which the study investigator and the participant are intentionally not informed (remain “blinded”) as to whether this participant received the vaccine candidate or placebo.

Clinical trials are designed to evaluate safety and efficacy based on a complete view of all participants and their experiences. Unexpected adverse events, including illnesses, can occur in study participants during any clinical study, especially large studies; they can occur in both vaccine and placebo groups and require evaluation. The full safety and efficacy results will be shared at the conclusion of the trial when we can present a complete assessment of the profile of our vaccine candidate to regulatory authorities for consideration.

In accordance with the clinical trial protocol and regulatory requirements, the Janssen study team remains blinded. This is to maintain the integrity of the data, which is essential to establish the safety and efficacy of the vaccine candidate.

Janssen’s Phase 3 ENSEMBLE COVID-19 Vaccine Candidate Clinical Trial
The Phase 3 ENSEMBLE trial is a randomized, double-blind, placebo-controlled clinical trial designed to evaluate the safety and efficacy of a single dose of a vaccine versus placebo in up to 60,000 adults 18 years old and older, including significant representation from those who are over age 60.

Johnson & Johnson will develop and test its COVID-19 vaccine candidate in accordance with high ethical standards and sound scientific principles, and we are also committed to

accurate medical information and protecting participant privacy. We plan to disclose clinical trial data in our COVID-19 trials once those data are presented or published at pre-specified milestones and will proactively disclose regulatory trial holds requested by health authorities.

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About the Janssen Pharmaceutical Companies
At Janssen, we're creating a future where disease is a thing of the past. We're the Pharmaceutical Companies of Johnson & Johnson, working tirelessly to make that future a reality for patients everywhere by fighting sickness with science, improving access with ingenuity, and healing hopelessness with heart. We focus on areas of medicine where we can make the biggest difference: Cardiovascular & Metabolism, Immunology, Infectious Diseases & Vaccines, Neuroscience, Oncology, and Pulmonary Hypertension. Learn more at www.janssen.com. Follow us at @JanssenGlobal. Janssen is one of the Janssen Pharmaceutical Companies of Johnson & Johnson.

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Source: https://www.jnj.com/our-company/johnson-johnson-prepares-to-resume-phase-3-ensemble-trial-of-its-janssen-covid-19-vaccine-candidate-in-the-us

Johnson & Johnson and U.S. Department of Health & Human Services Expand Agreement to Support Next Phase of COVID-19 Vaccine Candidate Research and Development

NEW BRUNSWICK, N.J., November 14, 2020 – Johnson & Johnson (the Company) announced the expansion to the partnership between its Janssen Pharmaceutical Companies (Janssen) and the Biomedical Advanced Research and Development Authority (BARDA), which is part of the Office of the Assistant Secretary for Preparedness and Response (ASPR) at the U.S. Department of Health and Human Services for the ongoing development of Janssen's investigational COVID-19 vaccine candidate.

Under the amendment, Janssen will commit approximately $604 million and BARDA will commit approximately $454 million to support the ongoing Phase 3 ENSEMBLE trial evaluating Janssen's investigational COVID-19 vaccine candidate as a single-dose in up to 60,000 volunteers worldwide.

Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson, said, "We greatly value the ongoing confidence and support of our investigational COVID-19 vaccine candidate development program. Combined with our own significant investment, this agreement has enabled our vital research and development and underscores the importance of public-private partnerships to tackle the worldwide COVID-19 pandemic."

This project has been funded in whole or in part with Federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, under OTA No. HHSO100201700018C.

Johnson & Johnson affirmed its commitment to develop and test its Janssen COVID-19 vaccine candidate in accordance with high ethical standards and sound scientific principles, as outlined in a pledge made by nine vaccine manufacturers earlier this year.

For more information on Johnson & Johnson's multi-pronged approach to combatting the pandemic, visit: www.jnj.com/coronavirus.

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About Johnson & Johnson
At Johnson & Johnson, we believe good health is the foundation of vibrant lives, thriving communities and forward progress. That's why for more than 130 years, we have aimed to keep people well at every age and every stage of life. Today, as the world's largest and most broadly-based healthcare company, we are committed to using our reach and size for good. We strive to improve access and affordability, create healthier communities, and put a healthy mind, body and environment within reach of everyone, everywhere. We are blending our heart, science and ingenuity to profoundly change the trajectory of health for humanity. Learn more at www.jnj.com. Follow us at @JNJNews.

About the Janssen Pharmaceutical Companies
At Janssen, we're creating a future where disease is a thing of the past. We're the Pharmaceutical Companies of Johnson & Johnson, working tirelessly to make that future a reality for patients everywhere by fighting sickness with science, improving access with ingenuity, and healing hopelessness with heart. We focus on areas of medicine where we can make the biggest difference: Cardiovascular & Metabolism, Immunology, Infectious Diseases & Vaccines, Neuroscience, Oncology, and Pulmonary Hypertension. Learn more at www.janssen.com. Follow us at @JanssenGlobal.

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Source: https://www.jnj.com/johnson-johnson-and-u-s-department-of-health-human-services-expand-agreement-to-support-next-phase-of-covid-19-vaccine-candidate-research-and-development

Johnson & Johnson Initiates Second Global Phase 3 Clinical Trial of its Janssen COVID-19 Vaccine Candidate

November 15, 2020 -- The Phase 3 ENSEMBLE study of the single-dose regimen of JNJ-78436735, the investigational vaccine candidate for the prevention of COVID-19 being developed by the Janssen Pharmaceutical Companies of Johnson & Johnson, continues to enroll and vaccinate study participants. ENSEMBLE is proceeding to enroll up to 60,000 participants worldwide.

In addition to the single-dose regimen ENSEMBLE study, Janssen has now initiated the two-dose regimen ENSEMBLE 2 trial. ENSEMBLE 2 is a complementary, planned, pivotal, large-scale, multi-country Phase 3 trial that will study the safety and efficacy of a two-dose regimen of the investigational Janssen vaccine candidate for the prevention of COVID-19 in up to 30,000 participants worldwide. The ENSEMBLE and ENSEMBLE 2 trials will run in parallel.

While a potentially safe and effective single-dose preventive COVID-19 vaccine would have significant benefits, particularly in a pandemic setting, Janssen's COVID-19 vaccine program has been designed to be extremely thorough and driven by science. As such, we are investigating multiple doses and dosing regimens to evaluate their long-term efficacy.

The Phase 3 ENSEMBLE and ENSEMBLE 2 trials follow positive interim results from the Company's ongoing Phase 1/2a clinical study, which is studying the safety profile and immunogenicity of both a single-dose and two-dose vaccination. The interim analysis showed that a single dose of the COVID-19 vaccine candidate induced a robust immune response and was generally well-tolerated.

PHASE 3 ENSEMBLE 2 STUDY

The Phase 3 ENSEMBLE 2 study (NCT04614948) is a randomized, double-blind, placebo-controlled clinical trial designed to evaluate the safety and efficacy of a two-dose vaccine regimen versus placebo in adults 18 years old and older with and without comorbidities associated with an increased risk for severe COVID-19. The study will assess efficacy of the investigational vaccine after both the first and second dose to evaluate protection against the virus and potential incremental benefits for duration of protection with a second dose.

Janssen will aim to enroll participants in Belgium, Colombia, France, Germany, the Philippines, South Africa, Spain, the United Kingdom and the United States. In order to evaluate the efficacy of Janssen's COVID-19 vaccine candidate, clinical trial sites in countries and areas with high incidence of COVID-19 and the ability to achieve a rapid initiation were selected.

ENSEMBLE 2 is being conducted in collaboration with the UK National Institute for Health Research (NIHR).

The Company is committed to transparency and sharing information related to the Phase 3 ENSEMBLE 2 study.

JANSSEN'S INVESTIGATIONAL COVID-19 VACCINE CANDIDATE

The Janssen COVID-19 vaccine candidate leverages the Company's AdVac® technology platform, which was also used to develop and manufacture Janssen's European Commission-approved Ebola vaccine and construct its Zika, RSV, and HIV investigational vaccine candidates. Janssen's AdVac® technology platform has been used to vaccinate more than 110,000 people to date across Janssen's investigational vaccine programs.

For more information on Johnson & Johnson's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

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Source: https://www.jnj.com/johnson-johnson-initiates-second-global-phase-3-clinical-trial-of-its-janssen-covid-19-vaccine-candidate

Johnson & Johnson Announces Initiation of Rolling Submission for its Single-dose Janssen COVID-19 Vaccine Candidate with the European Medicines Agency

Belgium – December 01, 2020. Janssen-Cilag International N.V., part of the Janssen Pharmaceutical Companies of Johnson & Johnson, has initiated a rolling submission with the European Medicines Agency (EMA) for its investigational single-dose vaccine candidate for the prevention of COVID-19.

The EMA's Committee for Medicinal Products for Human Use (CHMP) enabled a rolling review of the investigational single-dose Janssen COVID-19 vaccine candidate based principally on positive non-clinical data showing that the vaccine candidate elicits a robust immune response, as demonstrated by neutralizing antibodies. Janssen will continue to work in close collaboration with the EMA's CHMP to complete the rolling review process and to facilitate a conditional Marketing Authorisation Application (MAA) when appropriate.

In addition to the EMA, Janssen is in discussions with other regulatory authorities worldwide, as it prepares to initiate regulatory review processes for use of its investigational single-dose COVID-19 vaccine candidate during the pandemic response period.

Janssen is committed to bringing an affordable COVID-19 vaccine to the public on a not-for-profit basis for emergency pandemic use.

WHAT IS A ROLLING REVIEW?
A rolling review is a regulatory tool used by regulatory authorities to speed-up the assessment of potentially promising investigational medicines or vaccines during a public health emergency.

In normal circumstances, all data on an investigational vaccine's efficacy, safety, and quality, as well as all required documents, must be submitted together at the start of a license application procedure. However, in the case of a rolling review, a regulatory authority will review data as they become available from ongoing studies.

By reviewing data as they become available, the regulatory authority can reach its decision sooner on whether the vaccine should be authorized.

JANSSEN'S INVESTIGATIONAL COVID-19 VACCINE CANDIDATE
The investigational Janssen COVID-19 vaccine candidate leverages the Company's AdVac® vaccine platform, which was also used to develop and manufacture Janssen's European Commission-approved Ebola vaccine regimen and construct its Zika, RSV, and HIV investigational vaccine candidates. Janssen's AdVac® technology has been used to vaccinate more than 114,000 people to date across the Company's investigational vaccine programs.

The safety data profile from an interim analysis of the ongoing Phase 1/2a clinical trial of the investigational Janssen COVID-19 vaccine candidate – studying the safety profile and immunogenicity of both a single-dose and two-dose vaccination – is supportive of further development of the vaccine candidate. Immune responses were shown to be similar across the age groups studied, including older adults. Based on these initial findings, the investigational COVID-19 vaccine candidate is being further evaluated in the Phase 3 ENSEMBLE and ENSEMBLE 2 clinical trials.

For more information on the Company's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

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Source: https://www.janssen.com/johnson-johnson-announces-initiation-rolling-submission-its-single-dose-janssen-covid-19-vaccine-0

Johnson & Johnson Announces Its First Phase 3 COVID-19 Vaccine Trial ENSEMBLE is Fully Enrolled

United States – December 17, 2020. The large-scale, pivotal, multi-country Phase 3 trial (ENSEMBLE) of the investigational Janssen COVID-19 single-dose vaccine candidate is now fully enrolled with approximately 45,000 participants. Given the high incidence of COVID-19 among the general population in the countries where the trial is being conducted, this number of participants will be sufficient to generate the data needed to determine the efficacy and safety of the Company's investigational COVID-19 vaccine candidate. Johnson & Johnson expresses its thanks to all participants, trial sites and health care professionals involved in the ENSEMBLE study.

Interim data from the ENSEMBLE trial is currently anticipated to be available by the end of January 2021. However, as this trial is dependent on disease events, the timing is approximate. If the data indicate the vaccine is safe and effective, the Company expects to submit an Emergency Use Authorization application to the U.S. Food and Drug Administration (FDA) in February. Other health regulatory applications around the world will be made in parallel.

Johnson & Johnson continues to develop and test its COVID-19 vaccine candidate in accordance with high ethical standards and sound scientific principles. The Company is committed to transparency and sharing information relating to the Phase 3 ENSEMBLE study.

ENSEMBLE was initiated in collaboration with the Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response at the U.S. Department of Health and Human Services (HHS) under Other Transaction Agreement HHSO100201700018C, and the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) at HHS.

A separate Phase 3 clinical trial of the investigational Janssen COVID-19 vaccine candidate to explore a two-dose regimen of Janssen's vaccine candidate (ENSEMBLE 2) is ongoing.

For more information on the Company's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

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Source: https://www.janssen.com/johnson-johnson-announces-its-first-phase-3-covid-19-vaccine-trial-ensemble-fully-enrolled

Johnson & Johnson Announces Agreement in Principle with Gavi to Supply Janssen's COVID-19 Vaccine Candidate to Lower-Income Countries in 2021

United States – December 18, 2020. Janssen Pharmaceutica NV, one of the Janssen Pharmaceutical Companies of Johnson & Johnson (the Company), will provide up to 500 million doses of its investigational COVID-19 vaccine candidate as part of an agreement in principle with Gavi, The Vaccine Alliance (Gavi). Gavi is the leading multilateral organization responsible for equitable access to vaccines and coordination of procurement and distribution of COVID-19 vaccines, including to lower-income countries, via the COVAX Facility. These doses will be distributed through 2022, if the vaccine candidate is proven to be safe and effective.

The Company and Gavi expect to enter into an Advance Purchase Agreement (APA) that would provide the COVAX Facility with 100 million doses of Janssen's COVID-19 vaccine candidate in 2021, assuming the vaccine candidate receives regulatory approvals. Gavi also has the opportunity to order another 100 million doses in 2021, and up to 300 million doses in 2022, for a combined total of up to 500 million doses through 2022.

This collaboration is a part of the Company's commitment to ensuring widespread global access to its COVID-19 vaccine candidate on a not-for-profit basis for emergency pandemic use. In September 2020, Johnson & Johnson joined other life sciences companies and the Bill & Melinda Gates Foundation in signing an unprecedented communiqué which outlined an unwavering commitment to equitable access to the innovations being developed to fight the pandemic.

The COVAX Facility is a global risk-sharing mechanism, co-led by Gavi, for pooled procurement and equitable distribution of COVID-19 vaccines to all participating countries. The Facility is an important mechanism for ensuring equitable access in lower-income countries that can significantly increase their chances of securing successful vaccines. At this time, 190 countries have joined the COVAX Facility, including 92 low- and lower-middle-income countries.

Janssen's investigational COVID-19 vaccine candidate
The investigational Janssen COVID-19 vaccine candidate leverages the Company's AdVac® vaccine platform, which was also used to develop and manufacture Janssen's European Commission-approved Ebola vaccine regimen and construct its Zika, RSV, and HIV investigational vaccine candidates. Janssen's AdVac® technology has been used to vaccinate more than 150,000 people to date across the Company's investigational and approved vaccines.

The Company anticipates interim data from the Phase 3 ENSEMBLE study for its single-dose Janssen COVID-19 vaccine candidate to be available by the end of January 2021; however, as this trial is dependent on disease events, the timing is approximate. If the data indicate the vaccine is safe and effective, the Company expects to submit an

Emergency Use Authorization application to the U.S. Food and Drug Administration (FDA) in February, with other health regulatory applications around the world made in parallel.

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Source: https://www.janssen.com/johnson-johnson-announces-agreement-principle-gavi-supply-janssens-covid-19-vaccine-candidate-lower

Johnson & Johnson COVID-19 Vaccine Candidate Interim Phase 1/2a Data Published in *New England Journal of Medicine*

January 13, 2021 -- Interim Phase 1/2a data were published today in the *New England Journal of Medicine* demonstrating that the Company's single-dose investigational COVID-19 vaccine candidate (JNJ-78436735) – being developed by the Janssen Pharmaceutical Companies of Johnson & Johnson – provided an immune response that lasted for at least 71 days, the duration of time measured in this study in participants aged 18-55 years. A preview of part of these interim data was posted on *medRxiv* in September 2020.

The Phase 1/2a interim analysis showed that the Company's COVID-19 vaccine candidate induced an immune response and was generally well-tolerated across all study participants. Data demonstrated that, after a single vaccination, neutralizing antibodies against COVID-19 were detected in over 90 percent of study participants at Day 29 and 100 percent of participants aged 18-55 years at Day 57. These neutralizing antibodies remained stable through Day 71, currently the latest timepoint available in this ongoing study, in all participants aged 18-55 years. Data on durability of immune responses in trial participants aged over 65 years will be available in late January and longer-term follow-up to one year is planned.

The Company anticipates announcing topline Phase 3 data for its single-dose Janssen COVID-19 vaccine candidate in late January 2021; however, as this trial is dependent on disease events, the timing is approximate. If the single-dose vaccine is shown to be safe and effective, the Company expects to submit an application for Emergency Use Authorization with the U.S. Food and Drug Administration shortly afterwards, with other regulatory applications around the world to be made subsequently.

This Phase 1/2a study has been funded in whole or in part with Federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, under Contract No. HHSO100201700018C.

Phase 1/2a Study Design
This ongoing Phase 1/2a multi-center, randomized, double-blind, placebo-controlled trial aims to evaluate the safety, reactogenicity, and immunogenicity of Janssen's COVID-19 vaccine candidate at two dose levels ($5x10^{10}$ or $1x10^{11}$ viral particles), administered intramuscularly as single-dose or two-dose schedules, eight weeks apart, in healthy adults (aged 18 to 55 years; n=405 or >65 years; n=405). The study is ongoing at multiple clinical sites in Belgium and the United States.

The full set of results for this Phase 1/2a study will be published once the complete trial data are available.

Phase 1/2a Interim Analysis Safety Data
The interim analysis also included unblinded safety data which showed that injection site (local) and systemic reactions to vaccinations either occurred on the day of immunization or the next day, and generally resolved within 24 hours. The most frequent solicited adverse events (mild-to-moderate side effects typically associated with vaccinations) in the vaccine study arms were fatigue, headache, myalgia and injection site pain.

Reactogenicity was lower in the older age group. The study also evaluated a two-dose regimen, in which reactogenicity was observed to be lower after the second vaccine dose.

Five serious adverse events were reported; one participant visited the hospital for a fever that was associated with vaccination (the participant recovered within 12 hours); the remaining four were confirmed by study investigators as unrelated to the vaccine candidate.

Phase 1/2a Interim Analysis Immune Response Data
The interim data show that, following a single vaccination, neutralizing antibodies (VNA) titers – a laboratory test measuring the presence of antibodies in blood – against COVID-19 were detected in over 90 percent of participants at Day 29. In participants aged 18-55 years, this increased to 100 percent at Day 57 – irrespective of vaccine dose or age group. VNA titers then remained stable until at least Day 71 (currently the latest available time point in this ongoing study). Data on durability of immune responses in trial participants aged over 65 years after Day 29 will be shared in late January. The interim analysis showed the safety profile and immunogenicity after a single dose of the COVID-19 vaccine candidate were supportive of further development.

The study also evaluated a two-dose regimen, in which the data showed that a second dose of the vaccine candidate, administered 56 days apart, was less reactogenic while it triggered more than a two-fold increase in antibodies against COVID-19.

Janssen's Investigational COVID-19 Vaccine Candidate
The investigational Janssen COVID-19 vaccine candidate leverages the Company's AdVac® vaccine platform, which was also used to develop and manufacture Janssen's European Commission-approved Ebola vaccine regimen and construct its Zika, RSV, and HIV investigational vaccine candidates. Janssen's AdVac® technology has been used to vaccinate more than 200,000 people to date.

Janssen is investigating multiple doses and dosing regimens of its COVID-19 vaccine candidate to evaluate long-term efficacy. The Company is studying a single-dose of its vaccine candidate in the Phase 3 ENSEMBLE trial, which completed enrollment on December 17, 2020, and a two-dose regimen in the Phase 3 ENSEMBLE 2 study which is ongoing.

Johnson & Johnson continues to develop and test its COVID-19 vaccine candidate in accordance with ethical standards and sound scientific principles. The Company is committed to transparency and sharing information related to its ongoing clinical studies – including the ENSEMBLE study protocol.

ENSEMBLE was initiated in collaboration with the Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response at the U.S. Department of Health and Human Services (HHS) under Other Transaction Agreement HHSO100201700018C, and the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) at HHS.

For more information on the Company's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

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Source: https://www.jnj.com/johnson-johnson-covid-19-vaccine-candidate-interim-phase-1-2a-data-published-in-new-england-journal-of-medicine

Johnson & Johnson Announces Single-Shot Janssen COVID-19 Vaccine Candidate Met Primary Endpoints in Interim Analysis of its Phase 3 ENSEMBLE Trial

Vaccine Candidate 72% Effective in the US and 66% Effective Overall at Preventing Moderate to Severe COVID-19, 28 Days after Vaccination

85% Effective Overall in Preventing Severe Disease and Demonstrated Complete Protection Against COVID-19 related Hospitalization and Death as of Day 28

Protection Against Severe Disease Across Geographies, Ages, and Multiple Virus Variants, including the SARS-CoV-2 Variant from the B.1.351 Lineage[1] Observed in South Africa

Single-shot compatible with standard vaccine distribution channels provides important tool in pandemic setting

NEW BRUNSWICK, N.J., January 29, 2021 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced topline efficacy and safety data from the Phase 3 ENSEMBLE clinical trial, demonstrating that the investigational single-dose COVID-19 vaccine in development at its Janssen Pharmaceutical Companies met all primary and key secondary endpoints. The topline safety and efficacy data are based on 43,783 participants accruing 468 symptomatic cases of COVID-19.

The Phase 3 ENSEMBLE study is designed to evaluate the efficacy and safety of the Janssen COVID-19 vaccine candidate in protecting moderate to severe COVID-19, with co-primary endpoints of 14 days and 28 days following vaccination. Among all participants from different geographies and including those infected with an emerging viral variant, Janssen's COVID-19 vaccine candidate was 66% effective overall in preventing moderate to severe COVID-19, 28 days after vaccination. The onset of protection was observed as early as day 14. The level of protection against moderate to severe COVID-19 infection was 72% in the United States, 66% in Latin America and 57% in South Africa, 28 days post-vaccination.

"Johnson & Johnson embarked on the global effort to combat the COVID-19 pandemic a year ago, and has brought the full force of our capabilities, as well as tremendous public-private partnerships, to enable the development of a single-shot vaccine. Our goal all along has been to create a simple, effective solution for the largest number of people possible, and to have maximum impact to help end the pandemic," said Alex Gorsky, Chairman, Board of Directors and Chief Executive Officer, Johnson & Johnson. "We're

[1] The B.1.351 lineage also known as 501Y.V2 variant and 20H/501Y.V2 (formerly 20C/501Y.V2) is a variant of SARS-CoV-2, the virus that causes COVID-19

proud to have reached this critical milestone and our commitment to address this global health crisis continues with urgency for everyone, everywhere."

Prevention of severe disease; protection against COVID-related hospitalization and death
The vaccine candidate was 85 percent effective in preventing severe disease across all regions studied,[i] 28 days after vaccination in all adults 18 years and older. Efficacy against severe disease increased over time with no cases in vaccinated participants reported after day 49.

The Janssen COVID-19 vaccine candidate demonstrated complete protection against COVID-related hospitalization and death, 28 days post-vaccination. There was a clear effect of the vaccine on COVID-19 cases requiring medical intervention (hospitalization, ICU admission, mechanical ventilation, extracorporeal membrane oxygenation (ECMO), with no reported cases among participants who had received the Janssen COVID-19 vaccine, 28 days post-vaccination.

"These topline results with a single-shot COVID-19 vaccine candidate represent a promising moment. The potential to significantly reduce the burden of severe disease, by providing an effective and well-tolerated vaccine with just one immunization, is a critical component of the global public health response," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson. "A one-shot vaccine is considered by the World Health Organization to be the best option in pandemic settings, enhancing access, distribution and compliance. Eighty-five percent efficacy in preventing severe COVID-19 disease and prevention of COVID-19-related medical interventions will potentially protect hundreds of millions of people from serious and fatal outcomes of COVID-19. It also offers the hope of helping ease the huge burden placed on healthcare systems and communities."

In the study, the definition of severe COVID-19 disease included laboratory-confirmed SARS-CoV-2 and one or more of the following: signs consistent with severe systemic illness, admission to an intensive care unit, respiratory failure, shock, organ failure or death, among other factors. Moderate COVID-19 disease was defined as laboratory-confirmed SARS-CoV-2 and one or more of the following: evidence of pneumonia, deep vein thrombosis, shortness of breath or abnormal blood oxygen saturation above 93%, abnormal respiratory rate (≥20); or two or more systemic symptoms suggestive of COVID-19.

Protection was generally consistent across race, age groups, including adults over 60 years of age (N= 13,610), and across all variants and regions studied, including South Africa where nearly all cases of COVID-19 (95%) were due to infection with a SARS-CoV-2 variant from the B.1.351 lineage[ii].

Multi-continent Study Provides Clinical Data on Multiple Emerging Viral Mutations
The ENSEMBLE study results include efficacy against newly emerging strains of coronavirus, including some highly infectious variants present in the US, Latin America and South Africa. The Phase 3 ENSEMBLE trial is being conducted at the height of the COVID-19 pandemic in eight countries and three regions, at a time when disease spread has accelerated throughout the world resulting in people having increased exposure to the virus.

“These results are a testament to the extraordinary efforts of everyone involved in our COVID-19 vaccine candidate clinical program, and we are extremely grateful to the clinical trial staff and trial participants for their invaluable contributions,” said Mathai Mammen, M.D., Ph.D., Global Head, Janssen Research & Development. “Changing the trajectory of the pandemic will require mass vaccination to create herd immunity, and a single-dose regimen with fast onset of protection and ease of delivery and storage provides a potential solution to reaching as many people as possible. The ability to avoid hospitalizations and deaths would change the game in combating the pandemic.”

Trial participants of the phase 3 ENSEMBLE study continue to be followed for up to two years for assessments of safety and efficacy. Therefore, these data may be updated based on ongoing analysis. The comprehensive available data set will be submitted to a peer-reviewed journal in the coming weeks.

Phase 3 ENSEMBLE Study Safety Data
The analysis included a concurrent review of the available Phase 3 ENSEMBLE study safety data by the Data and Safety Monitoring Board (DSMB), an independent group of experts, that did not report any significant safety concerns relating to the vaccine. A review of adverse events indicated that a single-dose of Janssen’s COVID-19 vaccine candidate was generally well-tolerated.

The safety profile was consistent with other vaccine candidates using Janssen’s AdVac® technology among more than 200,000 people to date. Overall fever rates were 9% and Grade 3 fever 0.2%. Overall serious adverse events (SAEs) reported were higher in participants who received placebo as compared to the active vaccine candidate. No anaphylaxis was observed.

Janssen Vaccine Candidate Access and Distribution
The Company is committed to bringing an affordable COVID-19 vaccine on a not-for-profit basis for emergency pandemic use, pending regulatory authorizations.

In addition, the Janssen vaccine candidate is compatible with standard vaccine distribution channels. If authorized, Janssen’s single-dose vaccine candidate is estimated to remain stable for two years at -20°C (-4°F), at least three months of which can be at temperatures of 2-8°C (36°F–46°F). The Company will ship the vaccine using the same cold chain technologies it uses today to transport other innovative medicines.

The Company intends to file for U.S. Emergency Use Authorization (EUA) in early February and expects to have product available to ship immediately following authorization. It expects to share more information on specifics of deployment as authorizations are secured and contracts are finalized. The Company’s anticipated manufacturing timeline will enable it to meet its 2021 supply commitments, including those signed with governments and global organizations.

Phase 3 ENSEMBLE Study Design
The Phase 3 ENSEMBLE study is a randomized, double-blind, placebo-controlled clinical trial designed to evaluate the safety and efficacy of a single-dose vaccine versus placebo in adults 18 years old and older.

The ENSEMBLE study was designed to evaluate the safety and efficacy of the Janssen vaccine candidate in protecting against both moderate and severe COVID-19 disease, with assessment of efficacy as of day 14 and as of day 28 as co-primary endpoints.

Phase 3 ENSEMBLE Study Demographics
The trial, conducted in eight countries across three continents, includes a diverse and broad population including 34% (N= 14,672) of participants over age 60.

The study enrolled 44% (N=19,302) of participants in the United States, 41% (N=17,905) in Central and South America (Argentina, Brazil, Chile, Colombia, Mexico, Peru) and 15% (N=6,576) in South Africa.

Forty-five percent of participants are female, 55% male.

Among participants globally, 59% are White/Caucasian; 45% are Hispanic and/or Latinx; 19% are Black/African American; 9% are Native American and 3% are Asian. In the United States, 74% are White/Caucasian; 15% are Hispanic and/or Latinx; 13% are Black/African American; 6% are Asian and 1% are Native American.

Forty-one percent of participants in the study had comorbidities associated with an increased risk for progression to severe COVID-19 (overall 41%), obesity (28.5%), type 2 diabetes (7.3%), hypertension (10.3%), HIV (2.8%); also other immunocompromised participants were in the study.

Janssen's Vaccine Technology
The investigational Janssen COVID-19 vaccine candidate leverages the Company's AdVac® vaccine platform, which was also used to develop and manufacture Janssen's European Commission-approved Ebola vaccine regimen and construct its Zika, RSV, and HIV investigational vaccine candidates.

The Janssen AdVac® viral vector technology can induce potent and long-lasting humoral and cellular immune responses, enabling the pursuit of vaccines for disease targets that are currently unpreventable or untreatable.

Johnson & Johnson continues to develop and test its COVID-19 vaccine candidate in accordance with ethical standards and sound scientific principles. The Company is committed to transparency and sharing information related to its ongoing clinical studies – including the ENSEMBLE study protocol.

ENSEMBLE has been funded in whole or in part with Federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority (BARDA), under Contract No. HHSO100201700018C, and in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) at the U.S. Department of Health and Human Services (HHS).

Janssen has worked with BARDA since 2015 on innovative solutions for influenza, chemical, biological, radiation and nuclear threats and emerging infectious diseases such as Ebola. In February 2020, Janssen and BARDA began work on the development of a COVID-19 vaccine based on Janssen's AdVac® technology.

The Janssen Pharmaceutical Companies entered into a collaboration with the Beth Israel Deaconess Medical Center (BIDMC) to support the development of the preventive vaccine candidate for COVID-19.

Janssen's COVID-19 vaccine program has been designed to be thorough and driven by science. As such, the Company is also investigating immune responses for different doses and dosing regimens as well as studying a two-dose regimen of its COVID-19 vaccine candidate for efficacy in the Phase 3 ENSEMBLE 2 study.

For more information on the Company's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

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[i] https://www.jnj.com/coronavirus/covid-19-phase-3-study-clinical-protocol
[ii] The B.1.351 lineage also known as 501Y.V2 variant and 20H/501Y.V2 (formerly 20C/501Y.V2) is a variant of SARS-CoV-2, the virus that causes COVID-19

Source: https://www.jnj.com/johnson-johnson-announces-single-shot-janssen-covid-19-vaccine-candidate-met-primary-endpoints-in-interim-analysis-of-its-phase-3-ensemble-trial

Johnson & Johnson Announces Submission of Application to the U.S. FDA for Emergency Use Authorization of its Investigational Single-Shot Janssen COVID-19 Vaccine Candidate

Johnson & Johnson intends to distribute vaccine to the U.S. government immediately following authorization, and expects to supply 100 million doses to the U.S. in the first half of 2021

NEW BRUNSWICK, N.J., February 4, 2021 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced that Janssen Biotech, Inc., has submitted an application to the U.S. Food and Drug Administration (FDA) requesting Emergency Use Authorization (EUA) for its investigational single-dose Janssen COVID-19 vaccine candidate. The Company's EUA submission is based on topline efficacy and safety data from the Phase 3 ENSEMBLE clinical trial, demonstrating that the investigational single-dose vaccine met all primary and key secondary endpoints. The Company expects to have product available to ship immediately following authorization.

"Today's submission for Emergency Use Authorization of our investigational single-shot COVID-19 vaccine is a pivotal step toward reducing the burden of disease for people globally and putting an end to the pandemic," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer at Johnson & Johnson. "Upon authorization of our investigational COVID-19 vaccine for emergency use, we are ready to begin shipping. With our submission to the FDA and our ongoing reviews with other health authorities around the world, we are working with great urgency to make our investigational vaccine available to the public as quickly as possible."

The Company has initiated rolling submissions with several health agencies outside the U.S., and will submit a Conditional Marketing Authorisation Application (cMAA) with the European Medicines Agency in the coming weeks.

Manufacturing and Supply Chain Information
The Janssen investigational vaccine is compatible with standard vaccine distribution channels. If authorized, Janssen's investigational single-dose vaccine is estimated to remain stable for two years at -4°F (-20°C), at least three months of which can be stored in most standard refrigerators at temperatures of 36°F–46°F (2°-8°C). The Company will ship the vaccine using the same cold chain technologies it uses today to transport other innovative medicines.

Janssen's Investigational COVID-19 Vaccine
The Janssen investigational COVID-19 vaccine leverages the Company's AdVac® vaccine platform, which was also used to develop and manufacture Janssen's European Commission-approved Ebola vaccine regimen and construct its investigational Zika, RSV, and HIV vaccines. The safety profile observed was consistent with other investigational vaccines using Janssen's AdVac® technology among more than 200,000 people to date.

Phase 3 ENSEMBLE Study Design
The Phase 3 ENSEMBLE study is a randomized, double-blind, placebo-controlled clinical trial in adults 18 years old and older. The study was designed to evaluate the safety and efficacy of the Janssen investigational vaccine in protecting against both moderate and severe COVID-19 disease, with assessment of efficacy as of day 14 and as of day 28 as co-primary endpoints.

The trial, conducted in eight countries across three continents, includes a diverse and broad population.

Research and development activities for the investigational Janssen COVID-19 vaccine including the ENSEMBLE clinical trial and the delivery of doses for the U.S. has been funded in whole or in part with federal funds from the U.S. Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority (BARDA), under Contract No. HHSO100201700018C, and in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) at the U.S. Department of Health and Human Services (HHS).

Janssen has worked with BARDA since 2015 on innovative solutions for influenza, chemical, biological, radiation and nuclear threats and emerging infectious diseases such as Ebola. In February 2020, Janssen and BARDA began work on the development of a COVID-19 vaccine based on Janssen's AdVac® technology.

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Johnson & Johnson Announces Submission of European Conditional Marketing Authorisation Application to the EMA for its Investigational Single-Shot Janssen COVID-19 Vaccine Candidate

NEW BRUNSWICK, N.J., February 16, 2021 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced that Janssen-Cilag International N.V., has submitted a conditional Marketing Authorisation Application (cMAA) to the European Medicines Agency (EMA) seeking authorisation for its investigational single-dose Janssen COVID-19 vaccine candidate. The submission is based on topline efficacy and safety data from the Phase 3 ENSEMBLE clinical trial.

“Throughout Europe, there remains an urgent need for additional COVID-19 vaccines, and today’s submission is a significant step forward in ensuring the European Union has another option to help reduce the impact the pandemic has had in Europe and around the world,” said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer at Johnson & Johnson. “We stand ready to begin distributing our vaccine within the European Union in the second quarter of 2021.”

Once a conditional Marketing Authorisation has been granted, the Company must fulfill specific obligations within defined timelines, including the supply of additional data.

The Company announced in December it had initiated a rolling submission with the EMA for its investigational Janssen COVID-19 vaccine, enabling the EMA to review data as they become available. In addition, rolling submissions for the investigational single-dose COVID-19 vaccine have been initiated in several countries worldwide and with the World Health Organization (WHO). The Company filed for Emergency Use Authorization (EUA) in the United States on February 4, 2021.

Manufacturing and Supply Chain Information
The Janssen investigational vaccine is compatible with standard vaccine distribution channels. If authorized, Janssen’s investigational single-dose vaccine is estimated to remain stable for two years at -20°C (-4°F), at least three months of which can be stored in most standard refrigerators at temperatures of 2°-8°C (36°F–46°F).

Janssen’s Investigational COVID-19 Vaccine
The Janssen investigational COVID-19 vaccine leverages the Company’s AdVac® vaccine platform, which was also used to develop and manufacture Janssen’s European Commission-approved Ebola vaccine regimen and construct its investigational Zika, RSV, and HIV vaccines.

Phase 3 ENSEMBLE Study Design
The Phase 3 ENSEMBLE study is a randomized, double-blind, placebo-controlled clinical trial in adults 18 years old and older. The study was designed to evaluate the safety and efficacy of the Janssen investigational vaccine in protecting against both moderate and severe COVID-19 disease, with assessment of efficacy as of day 14 and as of day 28 as co-primary endpoints.

The trial, conducted in eight countries across three continents, includes a diverse and broad population.

For more information on the Company's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

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Source: https://www.jnj.com/johnson-johnson-announces-submission-of-european-conditional-marketing-authorisation-application-to-the-ema-for-its-investigational-single-shot-janssen-covid-19-vaccine-candidate

Johnson & Johnson Announces Submission to World Health Organization for Emergency Use Listing of Investigational Single-Shot Janssen COVID-19 Vaccine Candidate

NEW BRUNSWICK, N.J., February 19, 2021– Johnson & Johnson (NYSE: JNJ) (the Company) announced that Janssen-Cilag International N.V. has submitted for Emergency Use Listing (EUL) to the World Health Organization (WHO) for the investigational single-dose Janssen COVID-19 vaccine candidate. The data package delivered today includes interim efficacy and safety results from the Phase 3 ENSEMBLE clinical trial. The Company's rolling submission of clinical data to WHO is now complete.

"Our filing with the World Health Organization marks another important step in our effort to combat COVID-19 and also in our unwavering commitment to equitable access," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer of Johnson & Johnson. "If we are to end the global pandemic, life-saving innovations like vaccines must be within reach for all countries."

The EUL procedure streamlines the process by which new or unlicensed products can be assessed for use during public health emergencies by governments and UN procurement agencies. The EUL process expedites access to such products in many countries around the world and is also a prerequisite to supply vaccines to the new COVAX Facility, a global mechanism for pooled procurement and distribution of COVID-19 vaccines in 190 participating countries, including 92 lower-income countries.

In December 2020, the Company entered into an agreement in principle with Gavi, the Vaccine Alliance (Gavi) in support of the COVAX Facility. The Company and Gavi expect to enter into an Advance Purchase Agreement (APA) that would provide up to 500 million doses of the Janssen vaccine to COVAX through 2022.

Commitment to Equitable Access
Equitable access is at the forefront of Johnson & Johnson's COVID-19 response. The Company's single-dose vaccine candidate and its compatibility with standard vaccine distribution channels align with WHO's recommendations for medical interventions in a pandemic setting, which emphasize ease of distribution, administration and compliance.

The Company is committed to ensuring global access to its COVID-19 vaccine candidate on a not-for-profit basis during the acute phase of the pandemic. In September 2020, Johnson & Johnson joined other life sciences companies and the Bill & Melinda Gates Foundation in signing an unprecedented communiqué which outlined a steadfast commitment to facilitating equitable access to the innovations being developed to fight the pandemic.

Regulatory Filings
The Company filed for Emergency Use Authorization (EUA) in the United States on February 4, 2021 and submitted a Conditional Marketing Authorisation Application (cMAA) in the European Union on February 15, 2021. In addition, rolling submissions for

the investigational single-dose COVID-19 vaccine have been initiated in several countries worldwide. The Company will continue to provide data on an ongoing basis in support of WHO prequalification for the Janssen COVID-19 vaccine candidate.

Manufacturing and Supply Chain Information
The Janssen investigational vaccine is compatible with standard vaccine distribution channels. If authorized, Janssen's investigational single-dose vaccine is estimated to remain stable for two years at -20°C (-4°F), at least three months of which can be stored in most standard refrigerators at temperatures of 2°-8°C (36°F–46°F).

Janssen's Investigational COVID-19 Vaccine
The Janssen investigational COVID-19 vaccine leverages the Company's AdVac® vaccine platform, which was also used to develop and manufacture Janssen's European Commission-approved Ebola vaccine regimen and construct its investigational Zika, RSV, and HIV vaccines.

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Notice to Investors Concerning Forward-Looking Statements
This press release contains "forward-looking statements" as defined in the Private Securities Litigation Reform Act of 1995 regarding development of a potential preventive vaccine for COVID-19. The reader is cautioned not to rely on these forward-looking statements. These statements are based on current expectations of future events. If underlying assumptions prove inaccurate or known or unknown risks or uncertainties materialize, actual results could vary materially from the expectations and projections of the Janssen Pharmaceutical Companies, and/or Johnson & Johnson. Risks and uncertainties include, but are not limited to: challenges and uncertainties inherent in product research and development, including the uncertainty of clinical success and of obtaining regulatory approvals; uncertainty of commercial success; manufacturing difficulties and delays; competition, including technological advances, new products and patents attained by competitors; challenges to patents; product efficacy or safety concerns resulting in product recalls or regulatory action; changes in behavior and spending patterns of purchasers of health care products and services; changes to applicable laws and regulations, including global health care reforms; and trends toward health care cost containment. A further list and descriptions of these risks, uncertainties and other factors can be found in Johnson & Johnson's Annual Report on Form 10-K for the fiscal year ended December 29, 2019, including in the sections captioned "Cautionary Note Regarding Forward-Looking Statements" and "Item 1A. Risk Factors," and in the company's most recently filed Quarterly Report on Form 10-Q, and the company's subsequent filings with the Securities and Exchange Commission. Copies of these filings are available online at www.sec.gov, www.jnj.com or on request from Johnson & Johnson. None of the Janssen Pharmaceutical Companies nor Johnson & Johnson undertakes to update any forward-looking statement as a result of new information or future events or developments.

Source: https://www.jnj.com/johnson-johnson-announces-submission-to-world-health-organization-for-emergency-use-listing-of-investigational-single-shot-janssen-covid-19-vaccine-candidate

Johnson & Johnson COVID-19 Vaccine Authorized by U.S. FDA For Emergency Use - First Single-Shot Vaccine in Fight Against Global Pandemic

Data demonstrated protection against COVID-19 related hospitalization and death, across countries with different variants

Available on not-for-profit basis for emergency pandemic use

Shipping vaccine immediately, delivering more than 20 million doses to U.S. in March,

100 million doses in first half of 2021

NEW BRUNSWICK, N.J., February 27, 2021 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced that the U.S. Food and Drug Administration (FDA) has issued Emergency Use Authorization (EUA) for its single-dose COVID-19 vaccine, developed by the Janssen Pharmaceutical Companies of Johnson & Johnson, to prevent COVID-19 in individuals 18 years of age and older.

This decision was based on the totality of scientific evidence, including data from the Phase 3 ENSEMBLE study that demonstrated the vaccine was 85 percent effective in preventing severe disease across all regions studied, and showed protection against COVID-19 related hospitalization and death, beginning 28 days after vaccination.

The terms of the EUA allow use of the vaccine while more data are gathered. The Company plans to file for a Biologics License Application (BLA) with the FDA later in 2021.

“This milestone follows a year of incredible work by our dedicated teams and unprecedented collaboration with health leaders around the world – all of whom shared a goal of bringing a single-shot vaccine to the public,” said Alex Gorsky, Chairman and Chief Executive Officer at Johnson & Johnson. “We will do everything we can to help bring this pandemic to an end, in the United States and throughout the world.”

“We believe the Johnson & Johnson single-shot COVID-19 vaccine is a critical tool for fighting this global pandemic, particularly as it shows protection across countries with different variants. A vaccine that protects against COVID-19, especially against the most dire outcomes of hospitalization and death, will help ease the burden on people and the strain on health systems worldwide,” said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer, Johnson & Johnson. “We look forward to our continued efforts around the world as we collectively aim to change the trajectory of this global pandemic.”

Johnson & Johnson is committed to making its COVID-19 vaccine available on a not-for-profit basis for emergency pandemic use. The Company has begun shipping its COVID-19 vaccine and expects to deliver enough single-shot vaccines by the end of March to enable the full vaccination of more than 20 million people in the U.S. The Company plans to deliver 100 million single-shot vaccines to the U.S. during the first half of 2021. The U.S. government will manage allocation and distribution of the vaccine in the U.S. This will be prioritized according to the populations identified by the CDC's Advisory Committee on Immunization Practices (ACIP) guidelines.

Johnson & Johnson also recently announced its submission of a European Conditional Marketing Authorisation Application to the European Medicines Agency as well as its filing for an Emergency Use Listing (EUL) with the World Health Organization for its COVID-19 vaccine candidate. In addition, rolling submissions for the single-dose COVID-19 vaccine candidate have been initiated in several countries worldwide.

The EUA follows a unanimous vote by the U.S. FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) on February 26, 2021.

"We are thankful for the efforts of all those who have volunteered to participate in our clinical trials, our scientists, collaborators, clinical trial sites and investigators. Through the combined commitment of everyone involved, we have been able to discover, develop and manufacture a single-shot COVID-19 vaccine to protect people around the world," said Mathai Mammen, M.D., Ph.D., Global Head, Janssen Research & Development, Johnson & Johnson.

Manufacturing and Supply Chain Information
The Johnson & Johnson COVID-19 single-dose vaccine is compatible with standard vaccine storage and distribution channels with ease of delivery to remote areas. The vaccine is estimated to remain stable for two years at -4°F (-20°C), and a maximum of three months at routine refrigeration at temperatures of 36-46°F (2 to 8°C). The Company will ship the vaccine using the same cold chain technologies it uses today to transport treatments for cancer, immunological disorders and other medicines. The COVID-19 vaccine should not be re-frozen if distributed at temperatures of 36°F–46°F (2°-8°C).

Johnson & Johnson's COVID-19 Vaccine
The Company's COVID-19 vaccine leverages the AdVac® vaccine platform, a unique and proprietary technology that was also used to develop and manufacture Janssen's European Commission-approved Ebola vaccine regimen and construct its investigational Zika, RSV, and HIV vaccines.

The Janssen COVID-19 vaccine has not been approved or licensed by the U.S. Food and Drug Administration (FDA), but has been authorized by FDA through an Emergency Use Authorization (EUA) for active immunization to prevent Coronavirus Disease 2019 (COVID-19) in individuals 18 years of age and older. There is no FDA-approved vaccine to prevent COVID-19.

The FDA EUA Fact Sheet for Healthcare Providers Administering Vaccine (Vaccination Providers) and full EUA Prescribing Information are available at: www.janssenlabels.com/emergency-use-authorization/Janssen+COVID-19+Vaccine-HCP-fact-sheet.pdf.

Phase 3 ENSEMBLE Study Design
The Phase 3 ENSEMBLE study is a randomized, double-blind, placebo-controlled clinical trial in individuals 18 years of age and older. The study was designed to evaluate the safety and efficacy of the Company's vaccine candidate in protecting against both moderate and severe COVID-19 disease, with assessment of efficacy as of day 14 and as of day 28 as co-primary endpoints. The study enrolled a total of 43,783 participants.

The trial, conducted in eight countries across three continents, includes a diverse and broad population including 34 percent of participants over age 60.

The study enrolled 44 percent of participants in the United States. Seventy-four percent of participants in the U.S. are White/Caucasian; 15 percent are Hispanic and/or Latinx; 13 percent are Black/African American; 6 percent are Asian and 1 percent are Native American.

Forty-one percent of participants in the study had comorbidities associated with an increased risk for progression to severe COVID-19.

Research and development activities for the Company's COVID-19 vaccine, including the ENSEMBLE clinical trial and the delivery of doses for the U.S., has been funded in part with federal funds from the U.S. Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority (BARDA), under Contract No. HHSO100201700018C, and in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) at the U.S. Department of Health and Human Services (HHS).

Johnson & Johnson has worked with BARDA since 2015 on innovative solutions for influenza, chemical, biological, radiation and nuclear threats and emerging infectious diseases such as Ebola.

For more information on the Company's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

Authorized Use
The Janssen COVID-19 vaccine is authorized for use under an Emergency Use Authorization (EUA) for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 18 years of age and older.

Important Safety Information
WHAT SHOULD YOU MENTION TO YOUR VACCINATION PROVIDER BEFORE YOU GET THE JANSSEN COVID-19 VACCINE?
Tell the vaccination provider about all of your medical conditions, including if you:
· have any allergies
· have a fever
· have a bleeding disorder or are on a blood thinner
· are immunocompromised or are on a medicine that affects your immune system
· are pregnant or plan to become pregnant
· are breastfeeding
· have received another COVID-19 vaccine

WHO SHOULD NOT GET THE JANSSEN COVID-19 VACCINE?
You should not get the Janssen COVID-19 Vaccine if you:
· had a severe allergic reaction to any ingredient of this vaccine.

HOW IS THE JANSSEN COVID-19 VACCINE GIVEN?
The Janssen COVID-19 Vaccine will be given to you as an injection into the muscle. The Janssen COVID-19 Vaccine vaccination schedule is a single dose.

WHAT ARE THE RISKS OF THE JANSSEN COVID-19 VACCINE?
Side effects that have been reported with the Janssen COVID-19 Vaccine include:
· Injection site reactions: pain, redness of the skin, and swelling.
· General side effects: headache, feeling very tired, muscle aches, nausea, fever.

There is a remote chance that the Janssen COVID-19 Vaccine could cause a severe allergic reaction. A severe allergic reaction would usually occur within a few minutes to one hour after getting a dose of the Janssen COVID-19 Vaccine. For this reason, your vaccination provider may ask you to stay at the place where you received your vaccine for monitoring after vaccination. Signs of a severe allergic reaction can include:
· Difficulty breathing
· Swelling of your face and throat
· A fast heartbeat
· A bad rash all over your body
· Dizziness and weakness

These may not be all the possible side effects of the Janssen COVID-19 Vaccine. Serious and unexpected effects may occur. The Janssen COVID-19 Vaccine is still being studied in clinical trials.

WHAT SHOULD I DO ABOUT SIDE EFFECTS?
If you experience a severe allergic reaction, call 9-1-1, or go to the nearest hospital. Call the vaccination provider or your healthcare provider if you have any side effects that bother you or do not go away.

Report vaccine side effects to **FDA/CDC Vaccine Adverse Event Reporting System (VAERS)**. The VAERS toll-free number is 1-800-822-7967 or report online to https://vaers.hhs.gov/reportevent.html. Please include “Janssen COVID-19 Vaccine EUA” in the first line of box #18 of the report form. In addition, you can report side effects to Janssen Biotech, Inc. at 1-800-565-4008.

Cautions Concerning Forward-Looking Statements
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difficulties and delays; competition, including technological advances, new products and patents attained by competitors; challenges to patents; product efficacy or safety concerns resulting in product recalls or regulatory action; changes in behavior and spending patterns of purchasers of health care products and services; changes to applicable laws and regulations, including global health care reforms; and trends toward health care cost containment. A further list and descriptions of these risks, uncertainties and other factors can be found in Johnson & Johnson's Annual Report on Form 10-K for the fiscal year ended January 3, 2021, including in the sections captioned “Cautionary Note Regarding Forward-Looking Statements” and “Item 1A. Risk Factors,” and in the company’s most recently filed Quarterly Report on Form 10-Q, and the company’s subsequent filings with the Securities and Exchange Commission. Copies of these filings are available online at www.sec.gov, www.jnj.com or on request from Johnson & Johnson. None of the Janssen Pharmaceutical Companies nor Johnson & Johnson undertakes to update any forward-looking statement as a result of new information or future events or developments.

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Source: https://www.jnj.com/johnson-johnson-covid-19-vaccine-authorized-by-u-s-fda-for-emergency-usefirst-single-shot-vaccine-in-fight-against-global-pandemic

Johnson & Johnson Single-Shot COVID-19 Vaccine Granted Conditional Marketing Authorization by European Commission

Data have demonstrated vaccine protects against COVID-19 related hospitalization in broad geographic regions, including those with emerging variants[1]

Decision follows the European Medicines Agency recommendation of the J&J COVID-19 vaccine[2]

The Company aims to begin delivery of its vaccine to the EU in the second half of April and is committed to supply 200 million doses in 2021[3]

NEW BRUNSWICK, N.J., March 11, 2021 – Johnson & Johnson (NYSE: JNJ) (the Company) today announced that the European Commission (EC) has granted a Conditional Marketing Authorization (CMA) for its single-dose COVID-19 vaccine, developed by the Janssen Pharmaceutical Companies of Johnson & Johnson (Janssen), to prevent COVID-19 in individuals 18 years of age and older.

The CMA follows a Positive Opinion from the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP).[2] The CMA is valid in all 27 member states of the European Union (EU), plus Norway, Iceland and Liechtenstein.

Data from the Phase 3 ENSEMBLE study showed that the Johnson & Johnson COVID-19 vaccine was well tolerated and demonstrated a 67 percent reduction in symptomatic COVID-19 disease in participants who received the vaccine in comparison to participants given the placebo.[2] The onset of protection was observed from day 14 and was maintained 28 days post-vaccination.[1] The data also demonstrated the vaccine was 85 percent effective in preventing severe disease across all regions studied, and showed protection against COVID-19 related hospitalisation and death, beginning 28 days after vaccination.[1]

"For more than a year, we have been working around the clock – leveraging the scientific minds, scale and resources of our global organisation to bring forward a COVID-19 vaccine," said Alex Gorsky, Chairman and Chief Executive Officer at Johnson & Johnson. "We are thrilled with today's Conditional Marketing Authorization by the European Commission, which enables our single-dose vaccine to reach many more communities in need, as we continue to do everything we can to help bring an end to this pandemic."

Johnson & Johnson is committed to making its COVID-19 vaccine available on a not-for-profit basis for emergency pandemic use. The Company aims to begin delivery of its single dose COVID-19 vaccine to the EU in the second half of April and to supply 200 million doses to the EU,[3] plus Norway and Iceland in 2021.

“This vaccine is the result of more than a decade of investment in research and development and deep commitment by our scientists. We appreciate the collaboration and the support of the European Commission in this monumental effort,” said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer at Johnson & Johnson. “With this Conditional Marketing Authorization, we are proud to bring our single-shot vaccine to help protect millions of people across EU member states.”

In December 2020, the Company announced that Janssen initiated a rolling submission with the EMA for its single-dose COVID-19 vaccine candidate, enabling an expedited CHMP review process.[4] The COVID-19 vaccine candidate has also been filed for an Emergency Use Listing (EUL) with the World Health Organization.[5] Rolling submissions for our vaccine candidate have also been initiated in several countries worldwide.

“This latest major regulatory milestone would not have been possible without the hard work and dedication of everyone involved in our COVID-19 vaccine clinical trial programme, including our J&J team, our partners and study participants,” said Mathai Mammen, M.D., Ph.D., Global Head, Janssen Research & Development, Johnson & Johnson. “We are delighted by today’s announcement and remain fully committed to continuing our COVID-19 vaccine clinical programme as we strive to provide our single-dose COVID-19 vaccine to people all over the world.”

The Company received Emergency Use Authorization (EUA) in the United States on February 27,[6] following a unanimous vote by the U.S. Food and Drug Administration’s Vaccines and Related Biological Products Advisory Committee on February 26, 2021.[7] The Johnson & Johnson single-dose COVID-19 vaccine has also been granted Interim Order authorisation in Canada.[8]

Manufacturing and Supply Chain Information
The Johnson & Johnson COVID-19 single-dose vaccine is compatible with standard vaccine storage and distribution channels enabling delivery to remote areas.[6] The vaccine is estimated to remain stable for two years at -25 to -15°C, and a maximum of three months of which can be at routine refrigeration at temperatures of 2°-8°C.[9] The Company will ship the vaccine using the same cold chain technologies it uses today to transport other medicines.[6]

Johnson & Johnson’s COVID-19 Vaccine
The Johnson & Johnson COVID-19 vaccine leverages the AdVac® vaccine platform, a unique and proprietary technology that was also used to develop and manufacture Janssen’s European Commission-approved Ebola vaccine regimen and construct its investigational Zika, RSV, and HIV vaccines.[10]

Phase 3 ENSEMBLE Study Design
The Phase 3 ENSEMBLE study is a randomised, double-blind, placebo-controlled clinical trial in individuals 18 years of age and older.[11] The study was designed to evaluate the safety and efficacy of the Company’s vaccine candidate in protecting against both moderate and severe COVID-19 disease, with assessment of efficacy as of day 14 and as of day 28 as co-primary endpoints.[12] The study enrolled a total of 43,783 participants.[6]

The trial, conducted in eight countries across three continents,[11] includes a diverse and broad population including 34 percent of participants over age 60.[1] Forty-one percent of

participants in the study had comorbidities associated with an increased risk for progression to severe COVID-19.[1]

For more information on the Company's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

Cautions Concerning Forward-Looking Statements

This press release contains "forward-looking statements" as defined in the Private Securities Litigation Reform Act of 1995 regarding development of a potential preventive vaccine for COVID-19. The reader is cautioned not to rely on these forward-looking statements. These statements are based on current expectations of future events. If underlying assumptions prove inaccurate or known or unknown risks or uncertainties materialize, actual results could vary materially from the expectations and projections of the Janssen Pharmaceutical Companies, and/or Johnson & Johnson. Risks and uncertainties include, but are not limited to: challenges and uncertainties inherent in product research and development, including the uncertainty of clinical success and of obtaining regulatory approvals; uncertainty of commercial success; manufacturing difficulties and delays; competition, including technological advances, new products and patents attained by competitors; challenges to patents; product efficacy or safety concerns resulting in product recalls or regulatory action; changes in behavior and spending patterns of purchasers of health care products and services; changes to applicable laws and regulations, including global health care reforms; and trends toward health care cost containment. A further list and descriptions of these risks, uncertainties and other factors can be found in Johnson & Johnson's Annual Report on Form 10-K for the fiscal year ended January 3, 2021, including in the sections captioned "Cautionary Note Regarding Forward-Looking Statements" and "Item 1A. Risk Factors," and in the company's most recently filed Quarterly Report on Form 10-Q, and the company's subsequent filings with the Securities and Exchange Commission. Copies of these filings are available online at www.sec.gov, www.jnj.com or on request from Johnson & Johnson. None of the Janssen Pharmaceutical Companies nor Johnson & Johnson undertakes to update any forward-looking statement as a result of new information or future events or developments.

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REFERENCES

1. Janssen. Johnson & Johnson Announces Single-Shot Janssen COVID-19 Vaccine Candidate Met Primary Endpoints in Interim Analysis of its Phase 3 ENSEMBLE Trial. Available at: https://www.janssen.com/emea/sites/www_janssen_com_emea/files/johnson_johnson_announces_single-shot_janssen_covid-19_vaccine_candidate_met_primary_endpoints_in_interim_analysis_of_its_phase_3_ensemble_trial.pdf. Last accessed: March 2021.
2. European Medicines Agency. EMA recommends COVID-19 Vaccine Janssen for authorisation in the EU. Available at: https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-janssen-authorisation-eu. Last accessed: March 2021.
3. Janssen. Johnson & Johnson Announces European Commission Approval of Agreement to Supply 200 Million Doses of Janssen's COVID-19 Vaccine Candidate. Available at: https://www.janssen.com/emea/sites/www_janssen_com_emea/files/johnson_johnso

n_announces_european_commission_approval_of_agreement_to_supply_200_million_doses_of_janssens_covid-19_vaccine_candidate.pdf. Last accessed: March 2021.
4. Janssen. Johnson & Johnson Announces Initiation of Rolling Submission for its Single-dose Janssen COVID-19 Vaccine Candidate with the European Medicines. Available at: http://www.janssen.com/sites/www_janssen_com_emea/files/jj_announces_initiation_of_rolling_submission_for_its_single_dose_janssen_covid19_vaccine_candidate_with_the_ema.pdf. Last accessed: March 2021.
5. Janssen. Johnson & Johnson Announces Submission to World Health Organization for Emergency Use Listing of Investigational Single-Shot Janssen COVID-19 Vaccine Candidate. Available at: https://www.janssen.com/emea/sites/www_janssen_com_emea/files/jj_announces_submission_to_who_for_eul_of_investigational_single-shot_janssen_covid-19_vaccine_candidate_.pdf. Last accessed: March 2021.
6. Johnson & Johnson. Johnson & Johnson COVID-19 Vaccine Authorized by U.S. FDA For Emergency Use - First Single-Shot Vaccine in Fight Against Global Pandemic. Available at: https://www.jnj.com/johnson-johnson-covid-19-vaccine-authorized-by-u-s-fda-for-emergency-usefirst-single-shot-vaccine-in-fight-against-global-pandemic. Last accessed: March 2021.
7. Johnson & Johnson. Johnson & Johnson Single-Shot COVID-19 Vaccine Candidate Unanimously Recommended for Emergency Use Authorization by U.S. FDA Advisory Committee. Available at: https://www.jnj.com/johnson-johnson-single-shot-covid-19-vaccine-candidate-unanimously-recommended-for-emergency-use-authorization-by-u-s-fda-advisory-committee. Last accessed: March 2021.
8. Johnson & Johnson. Johnson & Johnson COVID-19 Vaccine Granted Authorization under Interim Order by Health Canada For Emergency Use. Available at: https://www.jnj.com/johnson-johnson-covid-19-vaccine-granted-authorization-under-interim-order-by-health-canada-for-emergency-use. Last accessed: March 2021.
9. Summary of Product Characteristics. COVID-19 Vaccine Janssen suspension for injection. March 2021.
10. Custers, J., Kim, D., et al. Vaccines based on replication incompetent Ad26 viral vectors: Standardized template with key considerations for a risk/benefit assessment. Vaccine. 2020.
11. ClinicalTrials.gov. A study of Ad26.COV2.S for the Prevention of SARS-CoV-2-Mediated COVID-19 in Adult Participants (ENSEMBLE). Available at: https://clinicaltrials.gov/ct2/show/NCT04505722. Last accessed: March 2021.
12. Johnson & Johnson. COVID-19 Phase 3 study clinical protocol. Available at: https://www.jnj.com/coronavirus/covid-19-phase-3-study-clinical-protocol. Last accessed: March 2021.

Source: https://www.jnj.com/johnson-johnson-single-shot-covid-19-vaccine-granted-conditional-marketing-authorization-by-european-commission

Johnson & Johnson Single-Shot COVID-19 Vaccine Granted Emergency Use Listing by the World Health Organization

Data have demonstrated vaccine protects against COVID-19 related hospitalization and death in broad geographic regions, including those with variants of significant concern[1]

Available on not-for-profit basis for emergency pandemic use

Compatible with standard vaccine storage, distribution channels, enabling delivery to remote areas

NEW BRUNSWICK, N.J., March 12, 2021– Johnson & Johnson (NYSE: JNJ) (the Company) today announced that the World Health Organization (WHO) has issued Emergency Use Listing (EUL) for its single-shot COVID-19 vaccine, developed by the Janssen Pharmaceutical Companies of Johnson & Johnson (Janssen), to prevent COVID-19 in individuals 18 years of age and older.

Data from the Phase 3 ENSEMBLE study showed that the Johnson & Johnson COVID-19 vaccine was well tolerated and demonstrated a 67 percent reduction in symptomatic COVID-19 disease in participants who received the vaccine in comparison to participants given the placebo. The onset of protection was observed from day 14 and was maintained 28 days post-vaccination.

The data also demonstrated the vaccine was 85 percent effective in preventing severe disease across all regions studied, and showed protection against COVID-19 related hospitalization and death across countries with different variants, beginning 28 days after vaccination.[1] Variants observed in an ongoing analysis in the ENSEMBLE study included the B.1.351 variant which was identified in 95 percent of the COVID-19 cases in South Africa.

“From the beginning of the pandemic, we have worked to develop and deliver a vaccine that could protect the health of people everywhere, and today’s milestone represents significant progress toward ensuring global access to our single-shot vaccine,” said Alex Gorsky, Chairman and Chief Executive Officer at Johnson & Johnson. “We are moving forward with urgency and purpose to meet our commitments to the global community as we do all we can to help end the pandemic.”

The EUL procedure streamlines the process by which new or unlicensed products can be assessed for use during public health emergencies by governments and United Nations procurement agencies. The EUL process expedites access to such products in many countries around the world and is also a prerequisite to supply vaccines to the new COVAX Facility, a global mechanism for pooled procurement and distribution of COVID-19 vaccines in 190 participating countries, including 92 lower-income countries.

"The WHO listing of our single-shot COVID-19 vaccine advances our pledge to help stem this pandemic and our unwavering commitment to equitable access," said Paul Stoffels, M.D., Vice Chairman of the Executive Committee and Chief Scientific Officer at Johnson & Johnson. "Achieving this important prerequisite for distributing our vaccine through the COVAX Facility which is co-led by Gavi is a major step forward in making our vaccine accessible for all."

In December 2020, Johnson & Johnson entered into an agreement in principle with Gavi, the Vaccine Alliance (Gavi) in support of the COVAX Facility. Johnson & Johnson and Gavi expect to enter into an Advance Purchase Agreement (APA) that would provide up to 500 million doses of the Company's vaccine to COVAX through 2022.[2]

"A single-shot COVID-19 vaccine that can be distributed and stored using established supply chains has the potential to be very meaningful in the face of this global pandemic," said Mathai Mammen, M.D., Ph.D., Global Head, Janssen Research & Development at Johnson & Johnson. "In addition, the clinical data shared with WHO that informed the Emergency Use Listing demonstrated protection against disease across countries with multiple variants."

Commitment to Equitable Access
Equitable access is at the center of Johnson & Johnson's COVID-19 response. The Johnson & Johnson single-shot vaccine candidate and its compatibility with standard vaccine distribution channels align with WHO's recommendations for medical interventions in a pandemic setting, which emphasize ease of distribution, administration, and compliance.

The Company is committed to ensuring global access to the Johnson & Johnson single-shot COVID-19 vaccine candidate on a not-for-profit basis for emergency pandemic use. In September 2020, Johnson & Johnson joined other life sciences companies and the Bill & Melinda Gates Foundation in signing an unprecedented communiqué which outlined a steadfast commitment to facilitating equitable access to the innovations being developed to fight the pandemic.[3]

Regulatory Filings
Johnson & Johnson received Emergency Use Authorization (EUA) in the United States on February 27[4] following a unanimous vote by the U.S. Food and Drug Administration's Vaccines and Related Biological Products Advisory Committee on February 26, 2021.[5] The Company's single-shot COVID-19 vaccine was also granted Interim Order authorization in Canada on March 5, 2021[6] and Conditional Marketing Authorization (CMA) in the European Union on March 11, 2021.[7]

Manufacturing and Supply Chain Information
The Johnson & Johnson COVID-19 single-shot vaccine is compatible with standard vaccine storage and distribution channels enabling delivery to remote areas.[4] The vaccine is estimated to remain stable for two years at -25 to -15°C, and a maximum of three months of which can be at routine refrigeration at temperatures of 2°-8°C.[4,8] This enables the vaccine to be shipped using the same cold chain technologies used to transport other medicines and vaccines in routine use.[4,9]

Johnson & Johnson's COVID-19 Vaccine
The Johnson & Johnson COVID-19 vaccine uses the AdVac® vaccine platform, a proprietary technology that was also used to develop and manufacture Janssen's

European Commission-approved Ebola vaccine regimen and construct its investigational Zika, RSV, and HIV vaccines.[6]

Phase 3 ENSEMBLE Study Design
The Phase 3 ENSEMBLE study is a randomized, double-blind, placebo-controlled clinical trial in individuals 18 years of age and older.[10] The study was designed to evaluate the safety and efficacy of the Company's vaccine candidate in protecting against both moderate and severe COVID-19 disease, with assessment of efficacy as of day 14 and as of day 28 as co-primary endpoints.[11] The study enrolled a total of 43,783 participants.

The trial, conducted in eight countries across three continents,[10] includes a diverse and broad population of which 34 percent of participants were over age 60.[1] Forty-one percent of participants in the study had comorbidities associated with an increased risk for progression to severe COVID-19.[1]

For more information on the Company's multi-pronged approach to helping combat the pandemic, visit: www.jnj.com/coronavirus.

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About Johnson & Johnson
At Johnson & Johnson, we believe good health is the foundation of vibrant lives, thriving communities and forward progress. That's why for more than 130 years, we have aimed to keep people well at every age and every stage of life. Today, as the world's largest and most broadly-based healthcare company, we are committed to using our reach and size for good. We strive to improve access and affordability, create healthier communities, and put a healthy mind, body and environment within reach of everyone, everywhere. We are blending our heart, science and ingenuity to profoundly change the trajectory of health for humanity. Learn more at www.jnj.com. Follow us at @JNJNews.

About the Janssen Pharmaceutical Companies of Johnson & Johnson
At Janssen, we're creating a future where disease is a thing of the past. We're the Pharmaceutical Companies of Johnson & Johnson, working tirelessly to make that future a reality for patients everywhere by fighting sickness with science, improving access with ingenuity, and healing hopelessness with heart. We focus on areas of medicine where we can make the biggest difference: Cardiovascular & Metabolism, Immunology, Infectious Diseases & Vaccines, Neuroscience, Oncology, and Pulmonary Hypertension. Learn more at www.janssen.com. Follow us at @JanssenGlobal.

Cautions Concerning Forward-Looking Statements
This press release contains "forward-looking statements" as defined in the Private Securities Litigation Reform Act of 1995 regarding development of a potential preventive vaccine for COVID-19. The reader is cautioned not to rely on these forward-looking statements. These statements are based on current expectations of future events. If underlying assumptions prove inaccurate or known or unknown risks or uncertainties materialize, actual results could vary materially from the expectations and projections of the Janssen Pharmaceutical Companies, and/or Johnson & Johnson. Risks and uncertainties include, but are not limited to: challenges and uncertainties inherent in product research and development, including the uncertainty of clinical success and of obtaining regulatory approvals; uncertainty of commercial success; manufacturing

difficulties and delays; competition, including technological advances, new products and patents attained by competitors; challenges to patents; product efficacy or safety concerns resulting in product recalls or regulatory action; changes in behavior and spending patterns of purchasers of health care products and services; changes to applicable laws and regulations, including global health care reforms; and trends toward health care cost containment. A further list and descriptions of these risks, uncertainties and other factors can be found in Johnson & Johnson's Annual Report on Form 10-K for the fiscal year ended January 3, 2021, including in the sections captioned "Cautionary Note Regarding Forward-Looking Statements" and "Item 1A. Risk Factors," and in the company's most recently filed Quarterly Report on Form 10-Q, and the company's subsequent filings with the Securities and Exchange Commission. Copies of these filings are available online at www.sec.gov, www.jnj.com or on request from Johnson & Johnson. None of the Janssen Pharmaceutical Companies nor Johnson & Johnson undertakes to update any forward-looking statement as a result of new information or future events or developments.

1 Janssen. Johnson & Johnson Announces Single-Shot Janssen COVID-19 Vaccine Candidate Met Primary Endpoints in Interim Analysis of its Phase 3 ENSEMBLE Trial. Available at: https://www.janssen.com/emea/sites/www_janssen_com_emea/files/johnson_johnson_announces_single-shot_janssen_covid-19_vaccine_candidate_met_primary_endpoints_in_interim_analysis_of_its_phase_3_ensemble_trial.pdf Last accessed: March 2021.

2 Johnson & Johnson. Johnson & Johnson Announces Agreement in Principle with Gavi to Supply Janssen's COVID-19 Vaccine Candidate to Lower-Income Countries in 2021. Available at: https://www.jnj.com/our-company/johnson-johnson-announces-agreement-in-principle-with-gavi-to-supply-janssens-covid-19-vaccine-candidate-to-lower-income-countries-in-2021. Last accessed: March 2021.

3 Johnson & Johnson. Johnson & Johnson Joins Other Companies in Signing a Landmark Communiqué on Expanded Global Access for COVID-19. Available at: https://www.jnj.com/latest-news/johnson-johnson-signs-communique-on-expanded-global-access-for-covid-19-vaccines. Last accessed: March 2021.

4 Johnson & Johnson. Johnson & Johnson COVID-19 Vaccine Authorized by U.S. FDA For Emergency Use - First Single-Shot Vaccine in Fight Against Global Pandemic. Available at: https://www.jnj.com/johnson-johnson-covid-19-vaccine-authorized-by-u-s-fda-for-emergency-usefirst-single-shot-vaccine-in-fight-against-global-pandemic. Last accessed: March 2021.

5 Johnson & Johnson. Johnson & Johnson Single-Shot COVID-19 Vaccine Candidate Unanimously Recommended for Emergency Use Authorization by U.S. FDA Advisory Committee. Available at: https://www.jnj.com/johnson-johnson-single-shot-covid-19-vaccine-candidate-unanimously-recommended-for-emergency-use-authorization-by-u-s-fda-advisory-committee. Last accessed: March 2021.

6 Johnson & Johnson. Johnson & Johnson COVID-19 Vaccine Granted Authorization under Interim Order by Health Canada For Emergency Use. Available at: https://www.jnj.com/johnson-johnson-covid-19-vaccine-granted-authorization-under-interim-order-by-health-canada-for-emergency-use. Last accessed: March 2021.

7 Johnson & Johnson. Johnson & Johnson Single-Shot COVID-19 Vaccine Granted Conditional Marketing Authorization by European Commission. Available at: https://www.jnj.com/johnson-johnson-single-shot-covid-19-vaccine-granted-conditional-marketing-authorization-by-european-commission

8 Custers, J., Kim, D., et al. Vaccines based on replication incompetent Ad26 viral vectors: Standardized template with key considerations for a risk/benefit assessment. Vaccine. 2020.

9 ClinicalTrials.gov. A study of Ad26.COV2.S for the Prevention of SARS-CoV-2-Mediated COVID-19 in Adult Participants (ENSEMBLE). Available at: https://clinicaltrials.gov/ct2/show/NCT04505722. Last accessed: March 2021.

10 ClinicalTrials.gov. A study of Ad26.COV2.S for the Prevention of SARS-CoV-2-Mediated COVID-19 in Adult Participants (ENSEMBLE). Available at: https://clinicaltrials.gov/ct2/show/NCT04505722. Last accessed: March 2021.

11 Johnson & Johnson. COVID-19 Phase 3 study clinical protocol. Available at: https://www.jnj.com/coronavirus/covid-19-phase-3-study-clinical-protocol. Last accessed: March 2021.

Source: https://www.jnj.com/johnson-johnson-single-shot-covid-19-vaccine-granted-emergency-use-listing-by-the-world-health-organization

Statement on the Interim SAGE Recommendation Supporting the Use of the Johnson & Johnson COVID-19 Vaccine

New Brunswick, NJ (March 17, 2021) – We welcome the interim recommendation by the Strategic Advisory Group of Experts (SAGE) on Immunization for the World Health Organization (WHO) supporting the use of Johnson & Johnson's single-shot COVID-19 vaccine, developed by the Janssen Pharmaceutical Companies of Johnson & Johnson, to prevent COVID-19 in persons aged 18 years and above. SAGE advises WHO on global vaccine and immunization policies and its recommendations for use provide guidance on the use of vaccines supplied through the COVAX Facility, a global risk-sharing mechanism for pooled procurement and equitable distribution of COVID-19 vaccines to all participating countries. At this time, 190 countries have joined the COVAX Facility, including 92 low- and lower-middle-income countries.

Johnson & Johnson is committed to ensuring global access to the Johnson & Johnson single-shot COVID-19 vaccine candidate on a not-for-profit basis for emergency pandemic use. In December 2020, Johnson & Johnson entered into an agreement in principle with Gavi, the Vaccine Alliance (Gavi), in support of the COVAX Facility. Johnson & Johnson and Gavi expect to enter into an Advance Purchase Agreement (APA) that would provide up to 500 million doses of the Company's vaccine to COVAX through 2022.

The SAGE recommendation was based on scientific evidence presented by Johnson & Johnson, including data from the Phase 3 ENSEMBLE study. Data from the Phase 3 ENSEMBLE study showed that the Johnson & Johnson COVID-19 vaccine was well tolerated and demonstrated a 67 percent reduction in symptomatic COVID-19 disease in participants who received the vaccine in comparison to participants given the placebo. The onset of protection was observed from day 14 and was maintained 28 days post-vaccination.

The data also demonstrated the vaccine was 85 percent effective in preventing severe disease across all regions studied, and showed protection against COVID-19 related hospitalization and death across countries with different variants, beginning 28 days after vaccination. Variants observed in an ongoing analysis in the ENSEMBLE study included the B.1.351 variant which was identified in 95 percent of the COVID-19 cases in South Africa.

Today's SAGE recommendation follows the WHO issuing Emergency Use Listing on March 12, the European Commission granting a Conditional Marketing Authorization on March 11, and Emergency Use Authorization issued by the U.S. Food and Drug Administration on February 27, 2021. The single-shot COVID-19 vaccine has also been granted Interim Order authorization in Canada, and additional rolling submissions have been initiated in several countries worldwide.

The Johnson & Johnson COVID-19 single-shot vaccine is compatible with standard vaccine storage and distribution channels enabling delivery to remote areas. The vaccine is estimated to remain stable for two years at -25° to -15°C, and a maximum of three months of which can be at routine refrigeration at temperatures of 2° to 8°C.

Therefore, it may be shipped using the same cold chain technologies used today to transport other medicines and vaccines that are in routine use.

The Johnson & Johnson COVID-19 vaccine leverages the AdVac® vaccine platform, a unique and proprietary technology that was also used to develop and manufacture the Company's European Commission-approved Ebola vaccine regimen and construct its investigational Zika, RSV, and HIV vaccines.

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Source: https://www.jnj.com/statement-on-the-interim-sage-recommendation-supporting-the-use-of-the-johnson-johnson-covid-19-vaccine