Australian Government

Department of Health

Therapeutic Goods Administration

Nonclinical Evaluation Report

ChAdOx1-S COVID-19 Vaccine [COVID-19 VACCINE ASTRAZENECA®]

Submission No: PM-2020-06115-1-2

Sponsor: AstraZeneca AB

January 2021

TGA Health Safety Regulation

NONCLINICAL EVALUATION REPORT

Submission type: New vaccine
Sponsor: AstraZeneca AB
Generic name: **ChAdOx1-S COVID-19 Vaccine**

Trade name: **COVID-19 VACCINE ASTRAZENECA**
Dose form and strength: Multi-dose vial contains 5 x10^{11} VP (ChAdOx1-S) in 5 mL
Vaccine Type: Recombinant (replication-defective chimpanzee adenovirus vector expressing the SARS CoV-2 S surface glycoprotein)

Submission No: PM-2020-06115-1-2
Tox file No: E21-210182
TRIM reference: D20-3849280

Date authorised: 28 January 2021

This report has been revised to incorporate the sponsor's s.31 response and replaces the Nonclinical Evaluation Report issued at Round 1

Note: This evaluation report has been peer-reviewed and is authorised for release to the sponsor.

TABLE OF CONTENTS

SUMMARY

- AstraZeneca Pty Ltd has applied for provisional registration of a new vaccine, ChAdOx1-S COVID-19 vaccine (also known as AZD1222). AZD1222 is a recombinant replication-defective chimpanzee adenovirus expressing the SARS-CoV-2 Spike (S) surface glycoprotein for the prevention of COVID-19 in persons 18 years of age and older. The proposed dosing regimen is two doses of 5×10^{10} VP/0.5 mL, given 4-12 weeks apart intramuscularly (IM).

- The sponsor has generally conducted adequate pharmacology studies. As part of a rolling submission limited toxicity studies were provided (which included a GLP compliant repeat dose) with AZD1222. A bio-distribution study and a main developmental and reproductive toxicity (DART) study in mice are pending.

- AZD1222 was found to be immunogenic in nonclinical studies in mice, ferrets, rhesus macaques and pigs. AZD1222 induced both humoral and cellular immune responses in the animal models (T cell response was not assessed in ferrets). Antibodies neutralised virus strains isolated at the beginning of the pandemic as well as the D614G mutant (no data on activity against the new variants isolated in the UK and South Africa, which are believed to be more transmissible). However, antibodies generally declined after 2 weeks of the booster dose, raising long term immunity concerns.

- The vaccine provided some protection in ferrets and monkeys when challenged 4 weeks after the 1st or 2nd dose based on lower viral load (in both models), milder lesions (in ferrets) and lower computed tomography (CT) scores (in macaques). Prime – boost vaccine regimen did not add to better protection in these studies, although a booster dose enhanced antibody titres and T cell responses. There were no studies on protection of older animals from SARS-CoV-2 infection or duration of protection after immunisation. The animal studies were of short term; long term immunity was not assessed.

- Anti-ChAd vector antibodies were detected in monkeys immunised with AZD1222. Immunogenicity of AZD1222 might decrease with repeated vaccination due to induction of anti-vector antibodies.

- No effects of AZD1222 on cardiovascular and respiratory parameters were observed in mice following vaccination with 2.59×10^{10} VP (>500 times the clinical dose on a mg/kg basis) by the clinical route (IM). Specific observations for neurological effects in the repeat dose toxicity study in mice revealed no vaccine-related findings.

- A distribution study with AZD1222 is ongoing. In a study with a vaccine consisting of the same chimpanzee adenovirus vector, ChAdOx1 as AZD1222 but expressing a hepatitis B virus antigen, the highest vector virus RNA was detected at the injection site, followed by draining lymph node and spleen, with low distribution to other tissues, and there was no virus shedding in urine or faeces.

- The ChAdOx1 vector is expected to have negligible risks of integrating into the human genome or recombination with human adenoviruses.

- No systemic toxicity was observed in repeat dose toxicity studies where mice were injected 3 doses of AZD1222 by the IM route, 14 days apart. Treatment-related findings were limited to (reversible) acute inflammation at the injection sites. Dosing for this study was adequate, where each dose was 1000 times the human dose of VP/kg ($\sim 1.23 \times 10^{12}$ VP/kg body weight in mice *cf.* 1×10^{9} VP/kg in 50 kg humans). Use of a single species (mice) is consistent with the relevant guidelines and demonstration of good immunogenicity supports the use of this species as an appropriate animal model for the toxicity study.

- A reproductive toxicity study with AZD1222 is currently ongoing.

- AZD1222 was well tolerated locally in mice, where local reactions due to AZD1222 were minimal inflammation.

CONCLUSIONS AND RECOMMENDATION

- Primary pharmacology studies indicate the vaccine elicits both neutralising antibody and cellular immune responses to the spike (S) antigen in animal models.
- Antibodies generally declined quickly over 2 weeks after the booster dose of AZD1222. Long term immunity was not assessed in nonclinical studies.
- Immunogenicity of AZD1222 might decrease with repeated vaccination due to induction of anti-vector antibodies.
- New variants are continuously emerging and require testing to confirm effective immunity and protection by AZD1222.
- The ChAdOx1 vector is expected to have negligible risks of integrating into the human genome or recombination with human adenoviruses.
- Repeat dose toxicity studies with the proposed vaccine in mice raised no safety issues. Treatment-related findings were limited to (reversible) acute inflammation at the injection sites.
- A reproductive toxicity study is currently ongoing and therefore Pregnancy category B2 is considered acceptable. Without adequate assessment of effects on embryofetal development, this vaccine is not recommended for use in pregnant women.
- There are no nonclinical objections to the provisional registration of the vaccine, provided efficacy of the prime-boost regimen has been satisfactorily addressed by clinical studies.
- The proposed non-clinical statements in the draft Product Information are acceptable.
- The following post approval commitments are to be provided by the sponsor as outlined in the Table below:

Issue	Commitment	Estimated timeline
Distribution study	To provide the AZD1222 tissue distribution study (Study 514559) report	Audited draft report 30MAR21 Final report 13APR21
Reproductive toxicity	To provide the AZD1222 development and reproductive study (Study 490843) report	Audited summary 1FEB21 Audited draft report 15FEB21 Final report 8MAR21

ASSESSMENT

AstraZeneca Pty Ltd has applied for provisional registration of a new vaccine, ChAdOx1-S COVID-19 vaccine, also known as AZD1222 (Trade name: COVID-19 Vaccine AstraZeneca), under section 23AA of the Act. AZD1222 is indicated for the prevention of COVID-19 in persons 18 years of age and older. AZD1222 is a recombinant replication-defective chimpanzee adenovirus expressing the SARS-CoV-2 Spike (S) surface glycoprotein driven by the human cytomegalovirus major immediate early promoter that includes intron A with a human tissue plasminogen activator leader sequence at the N terminus. The proposed dosing regimen is two doses of 5×10^{10} VP/ 0.5 mL, given 4-12 weeks apart intramuscularly (IM).

General comments

Module 4 comprised of nonclinical studies with clinical formulation. As a rolling submission, not all nonclinical data have been provided. The following data will be submitted later.

- AZD1222 (ChAdOx1-nCovd-19): A Single Dose Intramuscular Vaccine Biodistribution Study in the Mouse. Study Number 514556 (1169DM).
- A main developmental and reproductive toxicity (DART) study in mice.

Pharmacology

AZD1222 is a recombinant replication-defective chimpanzee adenovirus expressing the SARS-CoV-2 Spike (S) surface glycoprotein driven by the human cytomegalovirus major immediate early promoter that includes intron A with a human tissue plasminogen activator leader sequence at the N terminus.

Primary pharmacology

Pharmacology studies were done in mice (BALB/c, CD-1), ferrets, rhesus macaques and pigs, and were only designed to address short term immunity (i.e. neutralising antibodies (NAbs), T cell responses and antibody response following immunisation and challenge) generally for up to 28 days or less. Immunogenicity data from a booster dose (particularly NAbs) varied significantly among species, which made it difficult to relate to the likely clinical impact without further investigation. Prime – boost vaccine regimen did not add to better protection in these studies. Subtyping of cellular immune cells (such as memory T cells) were not provided. A T_H1-biased immune response was observed in mouse, and pigs (note, it was not investigated in ferrets and were limited in both the NHP studies). No evidence of vaccine-elicited disease enhancement were observed in any of the protection studies. There were no studies on protection of older animals from SARS-CoV-2 infection or duration of protection after immunisation. The immunisation doses in all animal studies (6×10^9 – 5.12×10^{10} VP/animal) were less than or equal to the clinical dose (5×10^{10} VP/subject) based on dose per subject.

Immunogenicity

Mouse studies: In mice (CD-1 & BALB/c) in the first study, a single IM dose (6×10^9 VP/animal) induced IgG (higher levels of IgG2a compared to IgG1), neutralising antibodies, IFN-γ and increased S-specific CD4+ and CD8+ T cell populations. In the second study, an IM booster dose (6×10^9 VP/animal) in mice given 4 weeks after the prime dose did not statistically increase T-cell responses compared with the prime only vaccination, 3 weeks after the prime or booster vaccination, and anti-S antibodies were significantly increased after the booster dose in only BALB/c mice, but not in CD-1 mice. Both studies showed that the cellular responses were predominantly IFN-γ+ and TNF-α+ positive T cells, with negligible frequencies of IL-4+ and IL-10+ cells, which together with findings of higher levels of IgG2a compared to IgG1 (in the first study) are suggestive of a T_H1-biased immune response.

Ferret studies: Ferrets vaccinated with a single IM dose of AZD1222 (2.5 x 10^{10} VP/animal) induced NAbs, which were detectable from day 7 and titres increased up to day 28 (last sampling time before challenge). In this cohort, NAbs were enhanced upon exposure to SARS-CoV-2 virus challenge (28 days post immunisation) and remained at significantly high levels 28 days post challenge (DPC). In contrast, in IM prime-boost vaccine studies (28 days intervals, with the same dose), a significant increase in NAbs was detected 7 days after the boost before rapidly decreased after another 7 days. The levels continued decreasing and no subsequent increase was detected upon exposure to the challenge (on day 56) up to day 70 (last sampling point). The limited, transient benefit of the booster dose was confirmed in a second study in ferrets (6285) where a similar trend with increases in NAbs for only a week followed by rapid decline was observed. Furthermore, no subsequent increase was detected upon exposure to the challenge virus. Cellular immunity was not investigated in the ferret studies.

Monkey studies: In both NHP studies, a single dose only (Study 6284) or a single dose followed by a booster, 4 weeks apart (Doremalen et al., 2020) by IM injection of AZD1222 (2.5 x 10^{10} VP/animal) induced variable levels of NAbs in all vaccinated animals, suggesting the vaccine is immunogenic. In the booster animals, S-specific and NAbs were significantly increased compared to prime dose on day 14; however, this declined rapidly within 2 weeks. Interestingly, no statistically significant difference was observed in the T cell response including IFNγ+ T cells compared to controls 4 weeks post vaccination before virus challenge. In addition, the IFN-γ+ T cell response in the animals administered the booster was slightly lower than that in the prime only animals, with both vaccinated groups higher than the control group. Cellular immune response kinetics after vaccination was not determined.

Pig studies: A small number of pigs (*n*=3) received one (prime) or two doses (prime-boost, 28 days later) of AZD1222 (with 5.12 × 10^{10} VP/animal). Study results show a trend towards higher humoral (NAbs) and cellular responses (IFN –γ, CD4+, CD8+ T cell) in animals vaccinated with the prime-boost regimen. This was the only study where addition of a booster dose of the vaccine induced a sustained NAb response, at least for a short period of time measured in the study (14 days).

Protection against infection

AZD1222 provided some protection against SARS-CoV-2 challenge in ferrets and monkeys.

SARS-CoV-2 replicates in ferrets, but only causes mild clinical diseases and respiratory system pathology in this species (Muñoz-Fontela *et al*. 2020). Following challenge (3 x 10^4 $TCID_{50}$, SARS-CoV-2), no histological abnormalities developed in vaccinated and control animals in the first study. A decrease in virus shedding was detected by qRT-PCR from nasal wash, oral swab and rectal swab samples. However, in the second study (Study 6285), mild pulmonary lesions were observed in control animals, with a reduced severity in animals vaccinated with AZD1222 and a slightly increased severity (i.e. exacerbation) in animals vaccinated with formalin-inactivated SARS-CoV-2 (5 x 10^6 PFU) 7 DPC. Subsequent measurements revealed no significant differences 13-14 days post-challenge between the control and vaccinated groups. Vaccination with AZD1222 delayed lung pathology, whilst the opposite effect was observed with formalin-inactivated SARS CoV-2. There was little difference measured between prime only and prime – booster responses indicating little benefit of administration of a booster vaccination. Challenge doses were not comparable between the two ferrets studies as different units of virus dose were used (PFU or $TCID_{50}$), but the most likely difference in susceptibility could be due to a higher challenge dose used in the second study. The level of NAbs raised in response to vaccination appeared to correlate with reduced viral RNA in the upper respiratory tract with little difference in viral RNA between responses in prime only and prime boost groups.

SARS-CoV-2 replicates in rhesus macaques causing pathological features of viral pneumonia and variable induction of mild clinical disease (Muñoz-Fontela *et al*. 2020). Following challenge (5 x 10^6 PFU of SARS-CoV-2) in the single dose vaccination study, CT scan revealed a lower CT score at day 5

in vaccinated animals compared to controls and viral RNA was significantly lower on 7 DPC in BAL of the vaccinated animals compared to controls. Subsequent measurements revealed no significant differences 12-14 days post-challenge between the control and vaccinated groups. No clear distinction regarding adverse findings were reported in the lung in the provided histopathology data between controls and vaccinated animals at 13/14 DPC. In the prime – boost study clinical scores, viral loads in tissues, BAL gRNA and sgRNA levels were similar in both prime only and prime – boost animals but significantly lower than controls, suggesting both immunisation regimens provided protection and the booster dose did not add to better protection. An unexpected and unexplained finding of significantly higher viral RNA load in the intestinal tissues of prime–boost vaccinated animals compared to prime-only-vaccinated animals or control animals at 7 DPC requires further investigation. Serum IFN-γ levels (Th-1 cytokine) were upregulated in prime-only vaccinated animals, but not in prime-boost or unvaccinated control animals after virus challenge, and serum IL-10 levels (Th-2 cytokine) were upregulated in unvaccinated controls and one prime-only monkey but not in any prime-boost animals (other cytokines, including TNF, IL-2 and IL-4, were unaffected in any groups). Together, both these NHP studies show some protection in vaccinated animals 5-7 DPC with no evidence of vaccine-elicited disease enhancement.

Is the vaccine effective on all variant SARS-CoV-2 viruses?

During the course of the pandemic, mutations have arisen in the viral Spike (S) protein that has become dominant amongst viruses sequenced from patient samples. The sponsor has provided evidence showing that D614G mutation in the SARS-CoV-2 spike protein does not adversely affect virus neutralisation by Abs induced by AZD1222. However, other new variants are continuously emerging and require further testing to confirm effective immunity and protection against these new strains.

Prime/boost dose regimens

The observed lack of clear boosting of antibody or protection responses following a second immunisation in the mouse, ferret and monkey models requires further investigation. As discussed by the sponsor there are many possibilities for these observations including a high antigen causing dose blunting boost, clonal deletion by triggering apoptosis of high avidity T cells, inducing tolerance in targeted T cells or leading to terminal differentiation and exhaustion of T cells. It should be noted the dose regimen i.e. two doses at an interval of 4 to 12 weeks for clinical use have not been assessed in nonclinical studies (all studies in animal models were done with a dosing interval of 4 weeks).

Sterilizing Immunity

Sterilizing immunity, commonly seen with adenovirus vectors could also have played a role in the potential adenoviral removal after boost, hence limiting the effect of the booster. Vector antibodies were detected in the macaque pharmacology study (Doremalen et al., 2020) and are well known to be immune-dominant. Immunogenicity of AZD1222 might decrease with repeated vaccination due to induction of anti-vector antibodies.

Risk of ChAdOx1 integration into human genome and recombination with other adenoviruses.

The ChAdOx1 vaccine vector was derived from chimpanzee adenovirus isolate Y25 (CHAdY25), which is similar to the human adenovirus (HAdV) and have been grouped with HAdV species E (Dicks et al., 2012 & Lange et al., 2019). CHAdY25, was genetically modified and incapacitated for replication by deletion of the essential E1 genes and non-essential E3 genes. The E4 gene was modified to allow growth of the virus in T-REx-293 cells (that provide E1 region), during manufacturing of the vaccine. SARS-CoV-2 spike protein was inserted, to induce relevant antibodies. With these modifications, AZD1222 cannot replicate in the host cells. Vaccine quality will need to be assessed by the quality evaluator to ensure the vaccine has not gained replication competency during

manufacture. AdV are generally not considered to be capable of genetically integrating into the host genome but stay as episomal DNA in the nucleus of host cells until the cell is destroyed.

The likelihood of complementation and recombination of AZD1222 with other adenoviruses have been assessed as very low by the Office of the Gene Technology Regulator and recombination generally only occurs within the same adenovirus species (OGTR 2020). Naturally occurring homologues (wild-type ChAdV) are only known to circulate in chimpanzees. Adenovirus (AdV) infects epithelial cells of the respiratory, gastrointestinal, conjunctiva and urinary tract tissues (Lynch *et al.*, 2016). Administration of ChAdOx1 VP by the IM route is unlikely to encounter wild type AdV in epithelial cells as the VPs are expected to be largely confined to the IM injection site and distribution mainly to the draining lymph nodes and spleen.

The inserted gene encodes the full length SARS-COV-2 spike protein, and does not add virulence to the AdV vector. There is low homology between E1 flanking regions of the AZD1222 and HAdVs. Site directed recombination was used to insert the SARS-COV-2 spike into the E1 gene of AZD1222 making it stable and further decreasing the likelihood of recombination.

Safety pharmacology

No effects of AZD1222 on cardiovascular and respiratory parameters were observed in mice following vaccination with 2.59 x 10^{10} VP (>500 times the clinical dose on a mg/kg basis) by the clinical route (IM). With no CNS effects (based on a negative Irwin score) in mice in the repeat dose study with AZD1222, these safety pharmacology studies are sufficient and acceptable as per WHO guideline on nonclinical evaluation of vaccines (WHO 2005).

Pharmacokinetics

[redacted]

Repeat-dose toxicity

[redacted]

[redacted]

[redacted]

Genotoxicity

No genotoxicity studies were conducted. This is in line with relevant guidelines.

Carcinogenicity

Carcinogenicity studies were not conducted. This is acceptable based on its duration of use, and applicable guidelines.

Reproductive toxicity

As AZD1222 is indicated for the active immunisation of individuals from the age of 18 years, this vaccine can be potentially administered to pregnant women and women with childbearing potential. A main fertility and embryofetal development study is ongoing and until the final report is made available, this vaccine is not recommended for use in pregnant women.

[redacted]

Pregnancy classification

The sponsor has proposed Pregnancy Category B2. Given animal reproductive studies have not been completed, this is considered appropriate.

Local tolerance

Local tolerance was assessed in the repeat dose toxicity study with AZD1222 and studies with ChAdOx1 MERS and Chikungunya vaccines. Local reactions were limited to minimal inflammation

(oedema, haemorrhage and mononuclear cell and mixed cell infiltration) at the injection site and in some animals extending to the sciatic nerve (no findings in nerve axons) and were reversible.

Comments on the Nonclinical Safety Specification of the Risk Management Plan

Key findings from nonclinical data are adequately identified in the Safety Specification (Part II, Module SII) of the Risk Management Plan (Version 1, of 21 December 2020).

PRODUCT INFORMATION

ROUND 1 EVALUATION — MILESTONE 3

The following comments refer to the draft Product Information document (Doc ID-004452129 v1).

4.6 FERTILITY, PREGNANCY AND LACTATION

Effects on fertility

The proposed text is acceptable:

> "It is unknown whether COVID-19 Vaccine AstraZeneca may impact fertility. No data are available."

Use in pregnancy

The sponsor proposes Pregnancy Category B2 and the following statement, which is acceptable:

> "There are a limited amount of data from the use of COVID-19 Vaccine AstraZeneca in pregnant women, or women who became pregnant after receiving the vaccine. The data are insufficient to inform on vaccine associated risk.
>
> Animal reproductive toxicity studies have not been completed.
>
> As a precautionary measure, vaccination with COVID-19 Vaccine AstraZeneca is not recommended during pregnancy. Use of COVID-19 Vaccine AstraZeneca in pregnant women should be based on an assessment of whether the benefits of vaccination outweigh the potential risks."

Use in lactation

The following proposed text does not contain any nonclinical data and therefore requires the clinical evaluator to comment on acceptability.

> "There are no or limited data from the use of COVID-19 Vaccine AstraZeneca in lactating women. A risk to breastfed newborns/infants cannot be excluded.
>
> As a precautionary measure, it is preferable to avoid vaccination with COVID-19 Vaccine AstraZeneca when breastfeeding."

5.1 PHARMACODYNAMIC PROPERTIES

Mechanism of action

Statements on the mechanism of action are supported by nonclinical data.

> "COVID-19 Vaccine AstraZeneca is a monovalent vaccine composed of a single recombinant, replication-deficient chimpanzee adenovirus (ChAdOx1) vector encoding the S glycoprotein of SARS-COV-2. Following administration, the S glycoprotein of SARS-COV-2 is expressed locally stimulating neutralizing antibody and cellular immune responses."

MAIN BODY OF REPORT

INTRODUCTION

BACKGROUND

AstraZeneca Pty Ltd has applied for provisional registration of a new biological entity, ChAdOx1-S also known as AZD1222 (Trade name: COVID-19 Vaccine AstraZeneca), under section 23AA of the Act. AZD1222 is indicated for the prevention of COVID-19 in persons 18 years of age and older. AZD1222 is a recombinant replication-defective chimpanzee adenovirus expressing the SARS-CoV-2 Spike (S) surface glycoprotein driven by the human cytomegalovirus major immediate early promoter that includes intron A with a human tissue plasminogen activator leader sequence at the N terminus. The proposed dosing regimen is two doses of 5×10^{10} VP/0.5 mL per dose given 4 to 12 weeks apart intramuscularly (IM).

RELATED VACCINES

AZD1222 is a first adenovirus vector vaccine to be registered in Australia. There currently is no vaccines registered for COVID-19.

RECOMBINANT SARS-COV-2 SPIKE PROTEIN GENE

SARS-CoV-2 S (GenBank entry MN908947, amino acids 2-1273) was codon optimized to improve expression in human cells. DNA encoding the human tissue plasminogen activator (tPA) signal sequence was fused upstream of the S protein coding sequence. The tPA leader sequence enhances immunogenicity (Alharbi et al., 2017).

[redacted]

VIRAL VECTOR CONSTRUCT AND PRODUCT FORMULATION

AZD1222 (CAS No.: 2420395-83-9; other name: ChAdOx1 nCoV-19) is a recombinant, replication-deficient (E1 and E3 deleted) chimpanzee adenovirus that encodes the SARS-CoV-2 (nCoV-19) spike protein with a tissue plasminogen activator (tPA) leader sequence.

[redacted]

[redacted]

[redacted]

AZD1222 is formulated as indicated in Table 1.1. The quantity per vial represents label claim value of 5 mL. Each vial also includes a target overfill of 1.1 to 1.5 mL. The pH of the solution is 6.6. There are no novel excipients.

Table 0.1. Product formulation

Ingredient	Function	[redacted]
AZD1222	Active	
L-Histidine	Buffer	
L-Histidine hydrochloride monohydrate	Buffer	
Sodium chloride	Tonicifier/stabilizer	
Magnesium chloride hexahydrate	Stabilizer	
Disodium edetate (dihydrate)	Stabilizer	
Sucrose	Tonicifier/stabilizer	
Ethanol (anhydrous)*	Stabilizer	
Polysorbate 80	Surfactant/stabilizer	
Water for Injection	Aqueous vehicle	

*** May be substituted with diluted ethanol manufactured from compendial ethanol**

OVERSEAS REGULATORY STATUS

A similar application has been made in the EU, UK, Canada, Switzerland and Singapore (all between September-October 2020). UK has granted authorisation approved via Regulation 174 for emergency supply (December 2020)

SCOPE OF NONCLINICAL DATA

Module 4 comprised of nonclinical studies with clinical formulations. As a rolling submission, not all nonclinical data have been provided. The following data will be submitted later.

- AZD1222 (ChAdOx1-nCovd-19): A Single Dose Intramuscular Vaccine Biodistribution Study in the Mouse. Study Number 514556 (1169DM)
- A main developmental and reproductive toxicity (DART) study in mice

PRIMARY PHARMACOLOGY

A summary of nonclinical pharmacology program is shown in the Table below.

Summary of nonclinical pharmacological program

Study objective	Animal model and test material	Study
Murine immunopotency for different batches	Balb/C , Single dose **IM** 5x10⁹ VP, ChAdOx1 nCoV-19 (ADZ 1222), [redacted]	INT-ChAdOxl nCoV-19-POT-001 & INT-ChAdOx1 nCoV19-POT-004 (Internal report)
Murine Immunogenicity	Balb/C and CD-1 mice Single dose, IM 6 x 10⁹ VP AZD1222	Van Doremalen et al. 2020
	Balb/C and CD-1 mice Day 0 and 28, IM 6 x 10⁹ VP AZD1222	AR001111 & Graham et al. 2020
Non-human Primate Immunogenicity and protection	Rhesus macaque Day 0 and 28, IM 2.5 x 10¹⁰ VP AZD1222 or ChAdOx1 GFP	Van Doremalen et al. 2020
Non-human Primate Immunogenicity and protection	Rhesus macaques Single dose, IM 2.5 x 10¹⁰ vp AZD1222	6284
Ferret Immunogenicity and protection	Ferret Single dose, IM or IN 2.5 x 10¹⁰ vp AZD1222 or ChAdOx1 GFP	20-01125
	Ferret Day 0 and 28, IM 5.12 × 10¹⁰ vp AZD1222 or or ChAdOx1 GFP	6285
Porcine Immunogenicity	White-Landrace-Hampshire cross-bred pigs Day 0 and 28, IM 5.12 × 10¹⁰ vp AZD1222	AR001111 & Graham et al. 2020
Effect of D614G mutation on NAbs	Sera from n=6 ferrets from Study 20-01125, day 35 or 45 post-prime dose used to test 2 D614 variants and 1 G614 variants	20-01700

MURINE IMMUNOGENICITY

[redacted]

[redacted]

Doremalen et al., 2020
Mice BALB/c, (n = 5) and outbred CD-1 (n = 8)
Age: at least 6 weeks
Route: **IM** with 1 single dose of AZD1222 (6 x 10^9 VP) or ChAdOx1 GFP (control vaccine)
Immunity assessment: Humoral and cellular immune response (9-14 days after vaccination)

- Total IgG titres, were detected against spike protein subunits S1 and S2 in all vaccinated mice (Figure 1a), with higher levels of IgG2a compared to IgG1 (Figure 2).
- Virus-specific NAbs (cytopathic assay using SARS-CoV-2 virus and Vero E6 cells) were detected in all mice vaccinated with ChAdOx1 nCoV-19 (Figure 1b). Higher amounts were detected in CD-1 mice compared to BALB/c.
- Splenic T-cell response measured by IFN-γ ELISpot and intracellular cytokine staining (ICS), was detected against peptides spanning the full length of spike construct (Figure 1c).
- ↑ CD4+& CD8+ T cells were observed in both strains after immunization with increased expression levels of IFNγ and TNF and low expression of IL-4 and IL-10.

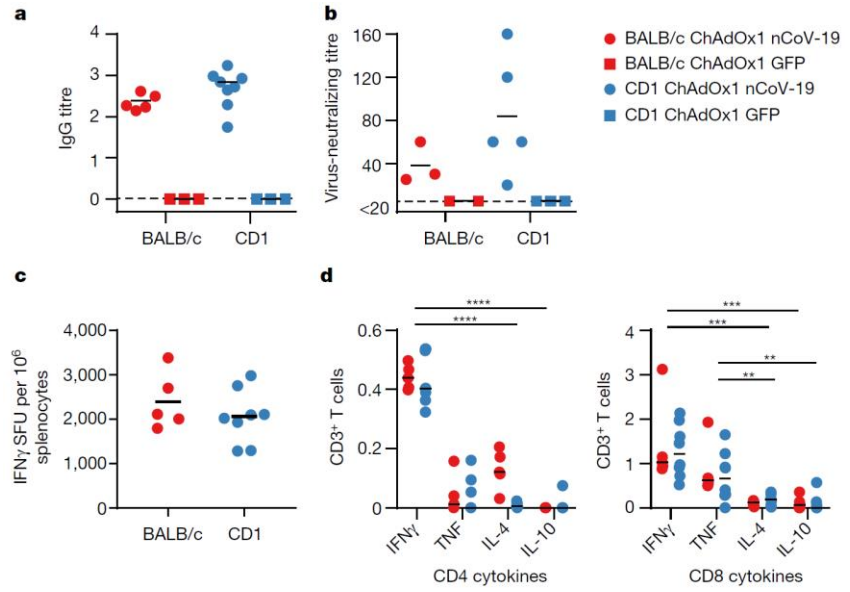


Figure 1. Humoral and cellular immune response to ChAdOx1 nCoV-19 vaccine in mice

a. End point titre of serum IgG detected against S protein on day 14. **b.** Virus neutralising titre in serum on day 9. **c.** Summed IFN-γ ELISpot responses in splenocytes towards peptides spanning the spike protein on day 14. Control mice had low (<100 SFU) or no detectable response. **d.** Summed frequency of spike-specific cytokine positive CD4+ or CD8+ T cells on day 14. SFU = spot-forming units.

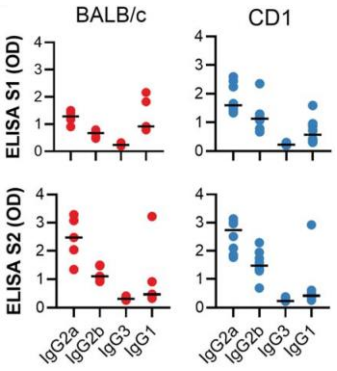
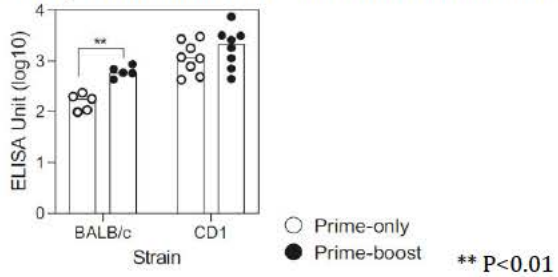


Figure 2. IgG subclass antibodies detected against S1 or S2 protein in sera of BALB/c or CD1 mice

Conclusion: A single dose of vaccine induced IgG (higher levels of IgG2a compared to IgG1), neutralising antibodies, increased S-specific CD4+ and CD8+ T cell populations in both species. Cellular responses were predominantly IFN-γ+ and TNF-α+ positive T cells, with negligible frequencies of IL-4+ and IL-10+ cells. Together these findings are suggestive of a TH1-biased immune response.

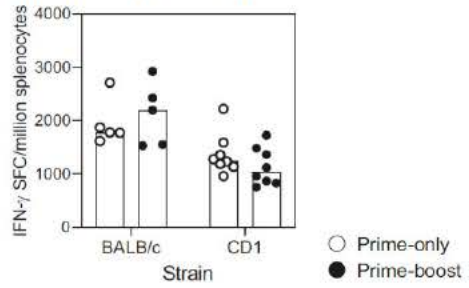
Report no. ar001111 & Graham et al. npj Vaccines. 2020;5:69. https://doi.org/10.1038/s41541-020-00221-3
Mice, inbred (BALB/c, *n* = 5/group) and outbred (CD1, *n*=8/group)
Age: 9-10 weeks
Immunisation: Day 0 (prime only) and 28 (prime and boost)
Route and dose: IM, 6 × 10^9 VP AZD1222
Assessment: Spleens & serum harvested on day 49.

- Antibodies were detectable by ELISA on Day 49 (see Figure below). Antibody titres increased after boost in BALB/c mice, but not in CD1 mice. Neutralisation antibodies were not determined.

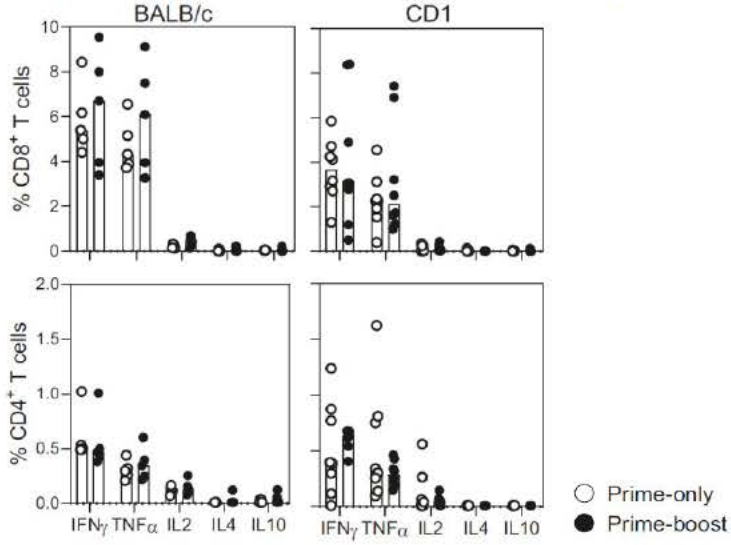


** P<0.01

- Analysis of SARS-CoV-2 S protein specific murine splenocyte responses by IFN-γ ELISpot assay showed no statistically significant difference between the prime only and prime-boost vaccination regimens, in either strain of mice (see figure below).



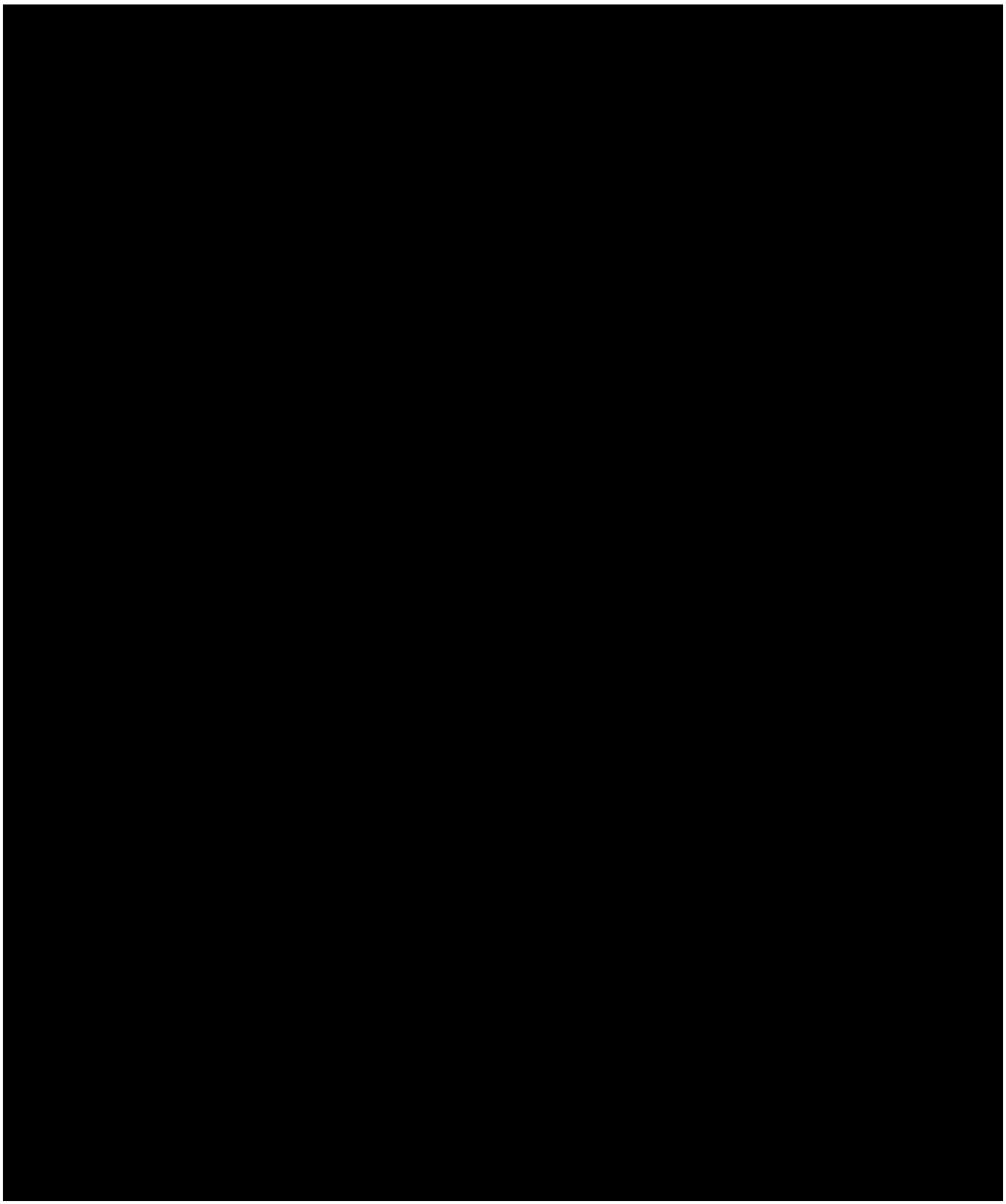
- Intracellular cytokine staining (ICS) of splenocytes (Figure below) showed that the cellular response was principally driven by CD8+ T cells.
- The predominant cytokine response of both CD8+ and CD4+ T cells was expression of IFN-γ and TNF-α, with negligible frequencies of IL-4+ and IL-10+ cells, suggesting a Th1 biased response.



Conclusion: IM booster dose did not statistically increase T-cell responses compared with the prime only vaccination, although anti-S antibodies were significantly increased after the booster dose in only BALB/c mice, but not in CD-1 mice. Cellular responses were predominantly IFN-γ+ and TNF-α+ positive T cells, with negligible frequencies of IL-4+ and IL-10+ cells, which is suggestive of a TH1-biased immune response.

FERRET IMMUNOGENICITY AND PROTECTION

[redacted]



Summary of prime and booster immunogenicity and protection studies in ferrets

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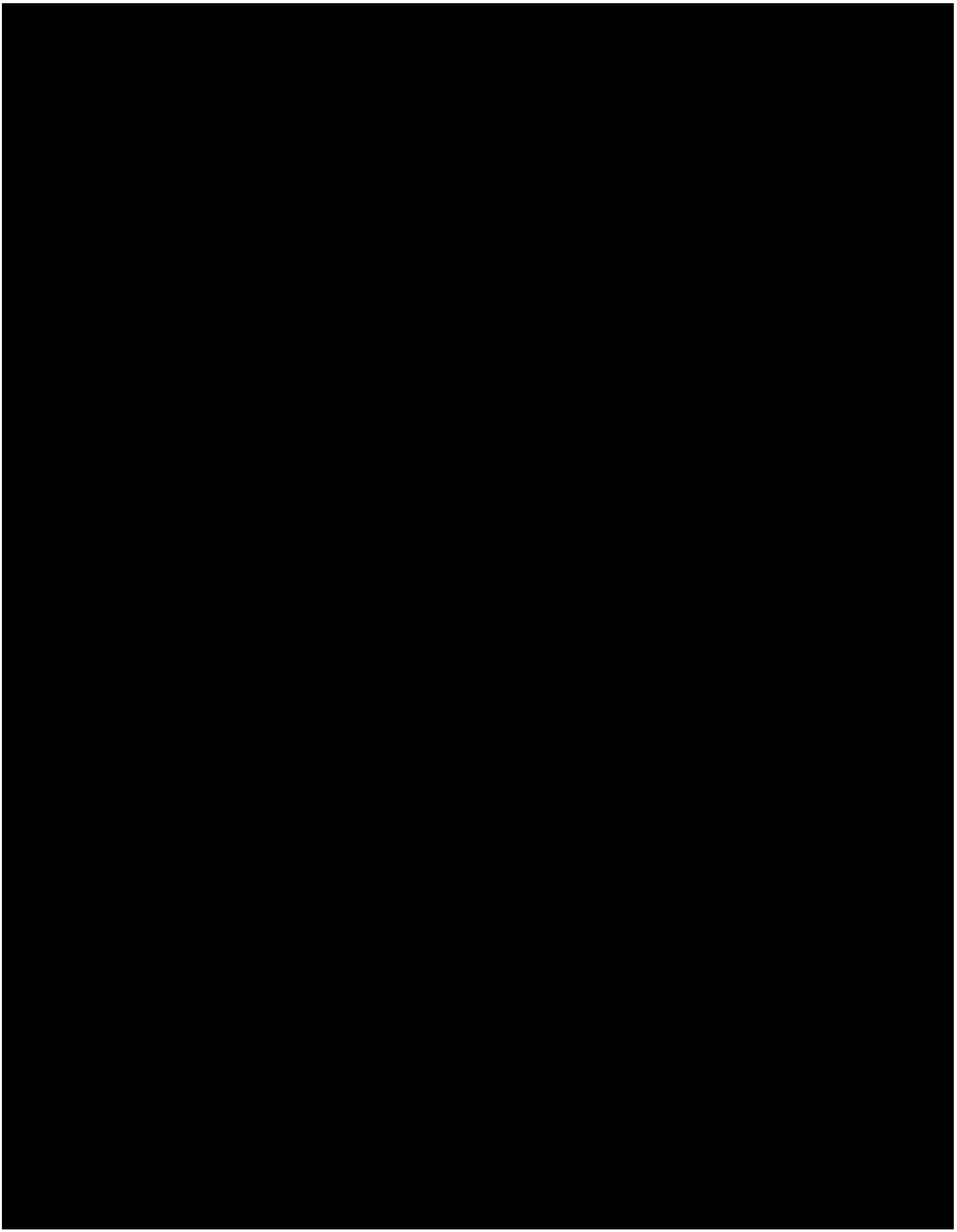

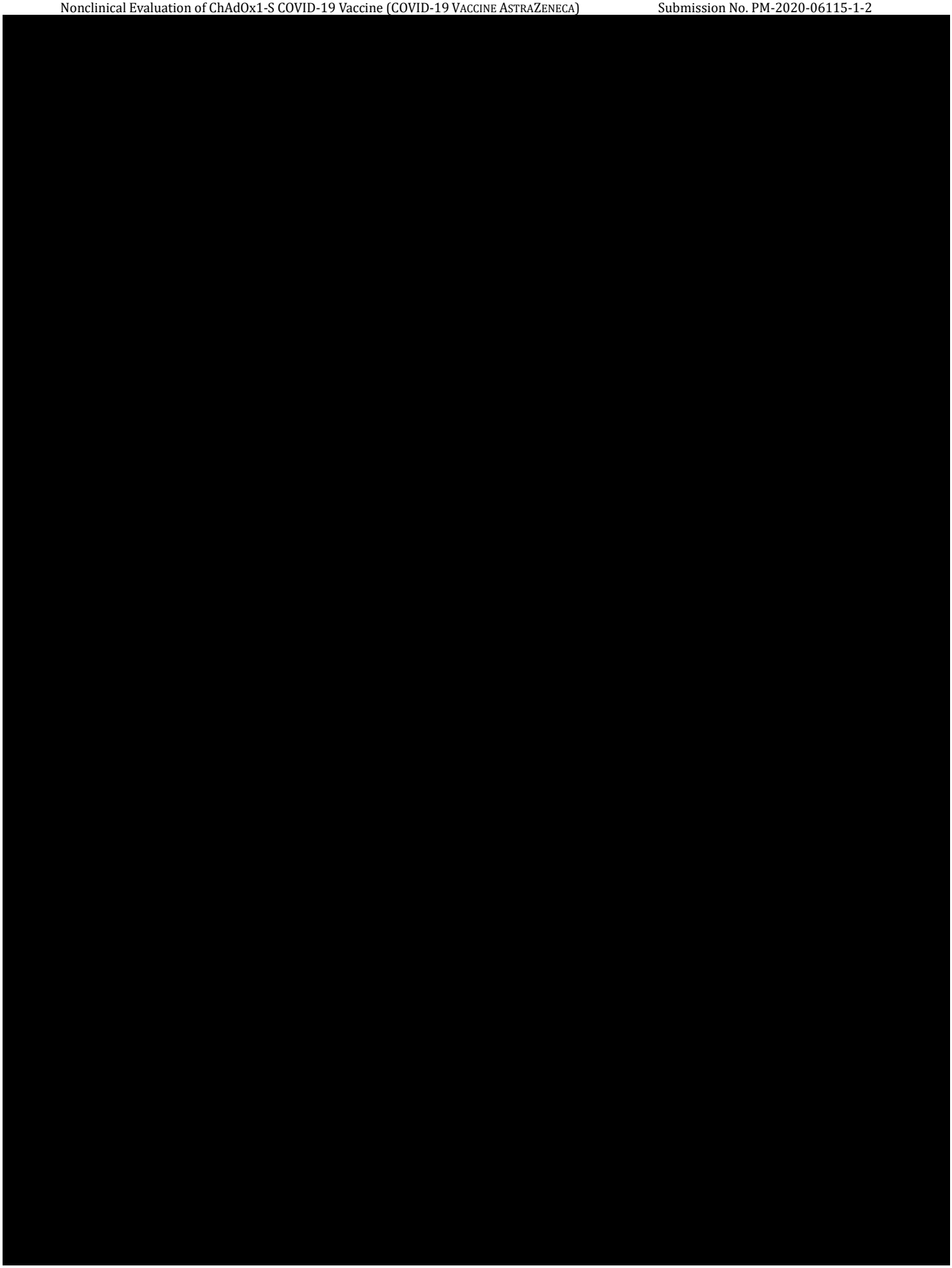

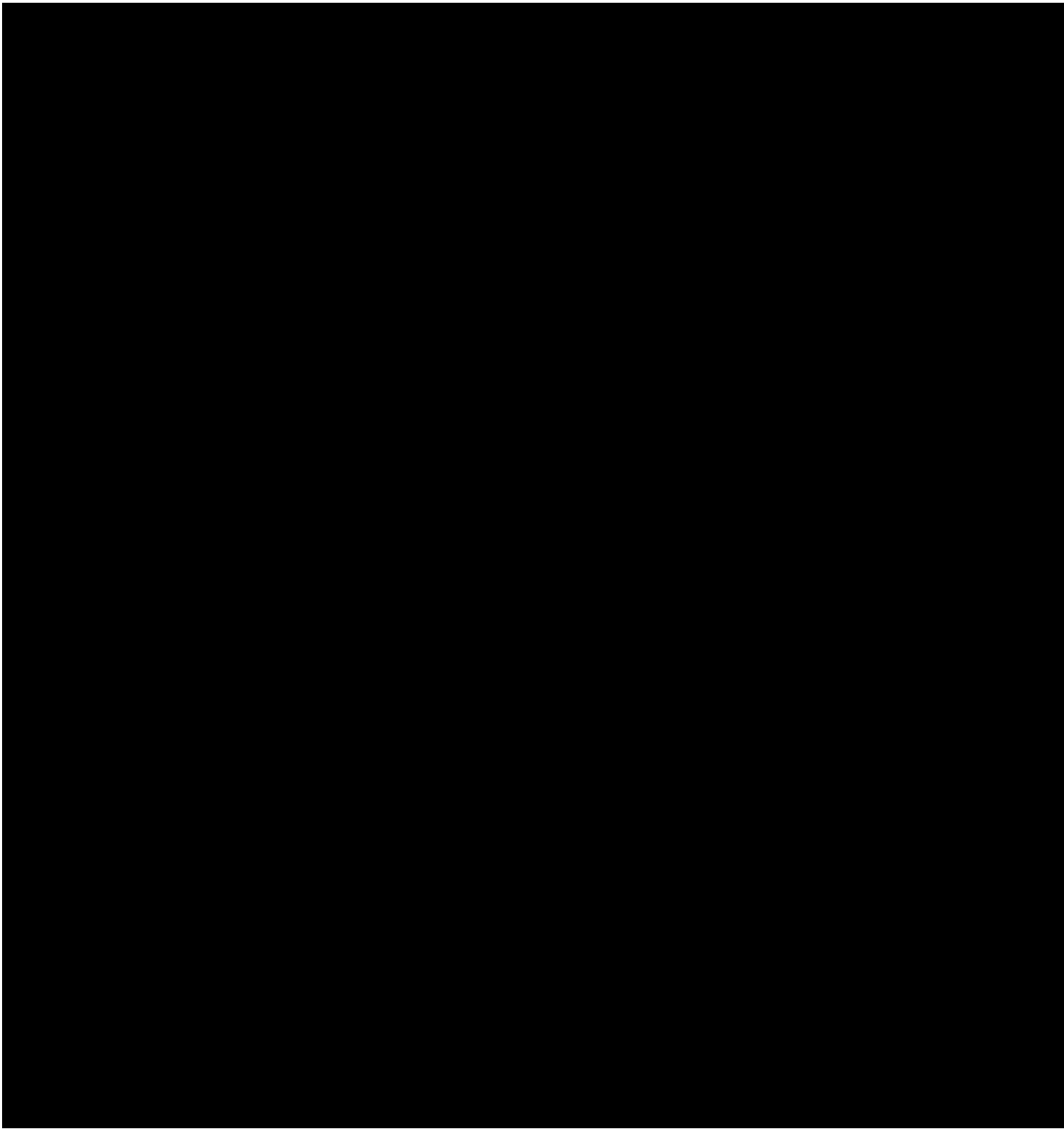

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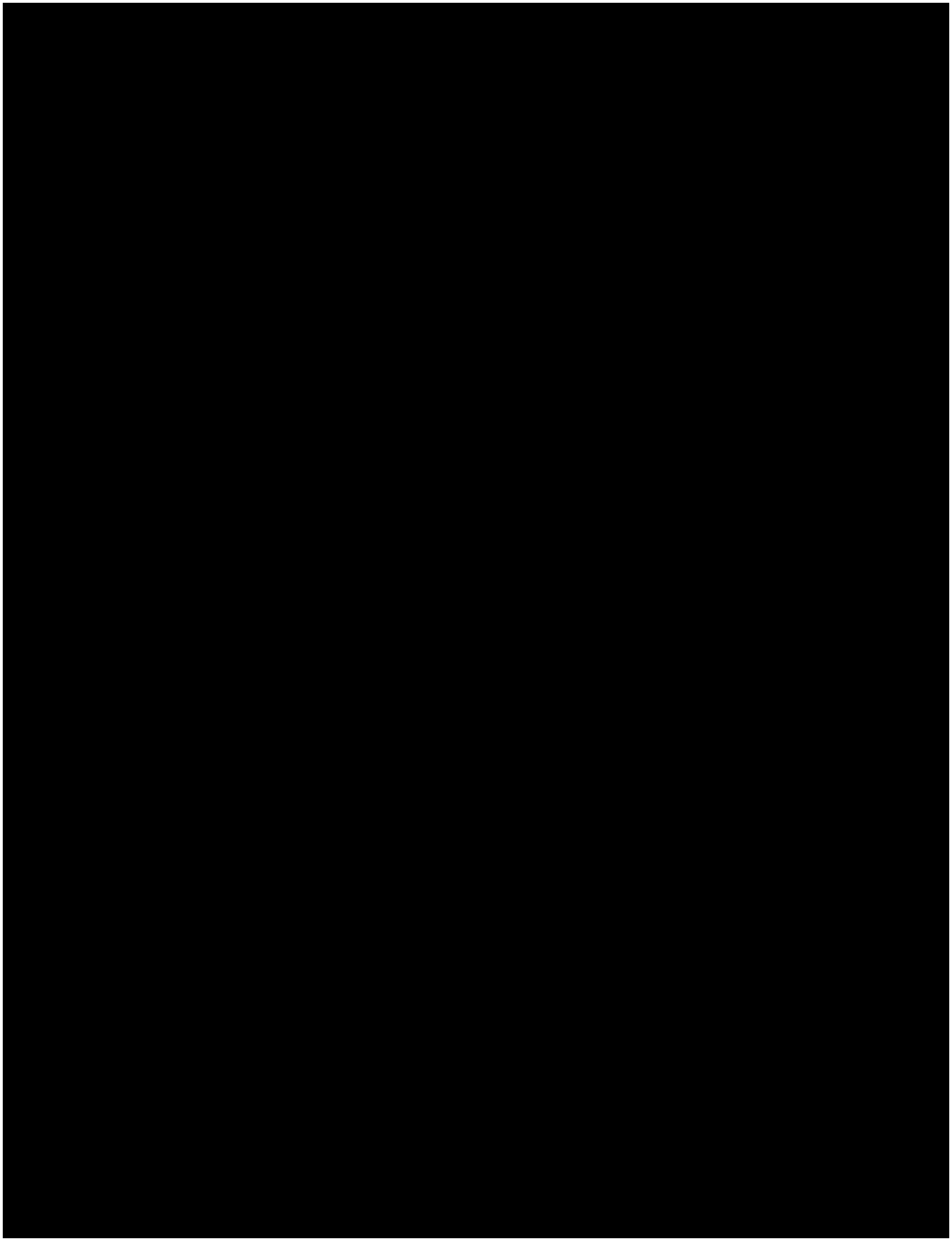
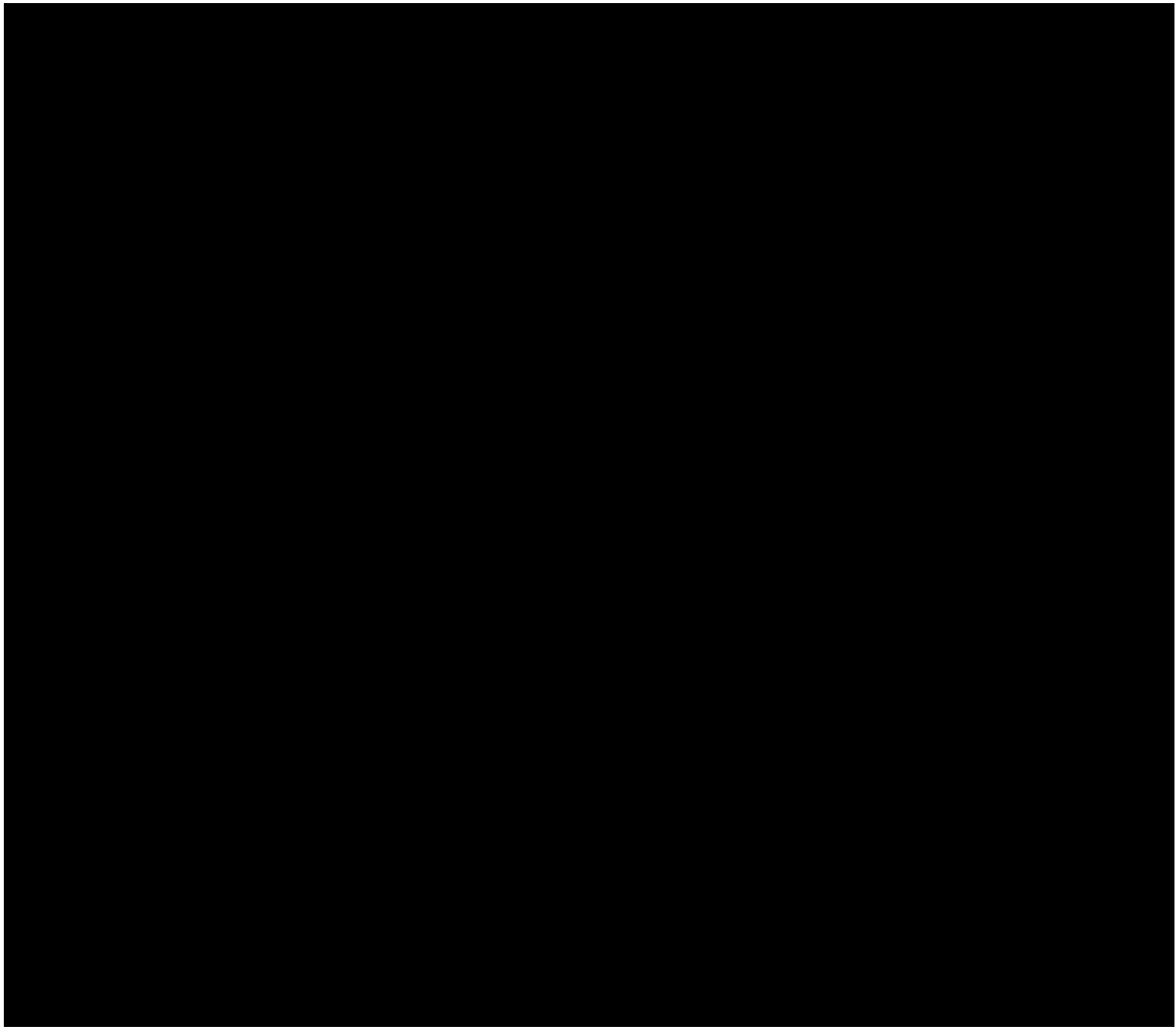




Study Details:
Doremalen et al., 2020
Rhesus macaques (17 male, 1 female; 2-4 years old)
Prime vaccination with 2.5 x 10^{10} VP AZD1222 (*n*=6) or ChAdOx1 GFP (*n* = 5) on day 0 via IM route.
Prime Boost vaccination with 2.5 x 10^{10} VP AZD1222 (*n*=6) or ChAdOx1 GFP (*n* = 1) at 4 weeks after 1st dose via IM route.
Challenge: 28 days post vaccination with 2.6 × 10^6 $TCID_{50}$ virus (strain nCoV-WA1-2020 (MN985325.1))
Challenge Route: IN (0.5 mL per nostril); IT (4 mL); oral (1 mL) and ocular (0.25 mL per eye) of 4 × 10^5 $TCID_{50}$ /mL in sterile DMEM
Animals were examined on 1, 3, 5, and 7 DPC and were euthanized at 7 DPC.
NAb assay: SARS-CoV-2 (strain not specified) in Vero E6 cells

Results

Adverse effects of vaccine

- No adverse events were observed after vaccination.

Immune response

- Spike-specific Abs were present as early as 14 days after vaccination and were significantly increased after the booster dose. Endpoint IgG titres of 400–6,400 (prime) and 400–19,200 (prime–boost) were measured on the day of challenge (Figure 2b)
- Virus-specific NAbs were also significantly increased after booster dose (day 14 after booster) and detectable in all vaccinated animals before challenge (5–40 (prime) and 10–160 (prime–boost)) (Figure 2c). However, the levels decreased before challenge (28 days after booster dose).

  SARS-CoV-2 spike-specific T cell responses were detected on the day of challenge by IFNγ ELISpot assay after the stimulation of PBMCs with a peptide library that spanned the full length of the spike protein. No statistically significant difference in the magnitude of the response was found between the prime–boost and prime-only group (Mann–Whitney U-test, P = 0.3723), (Figure 2d).

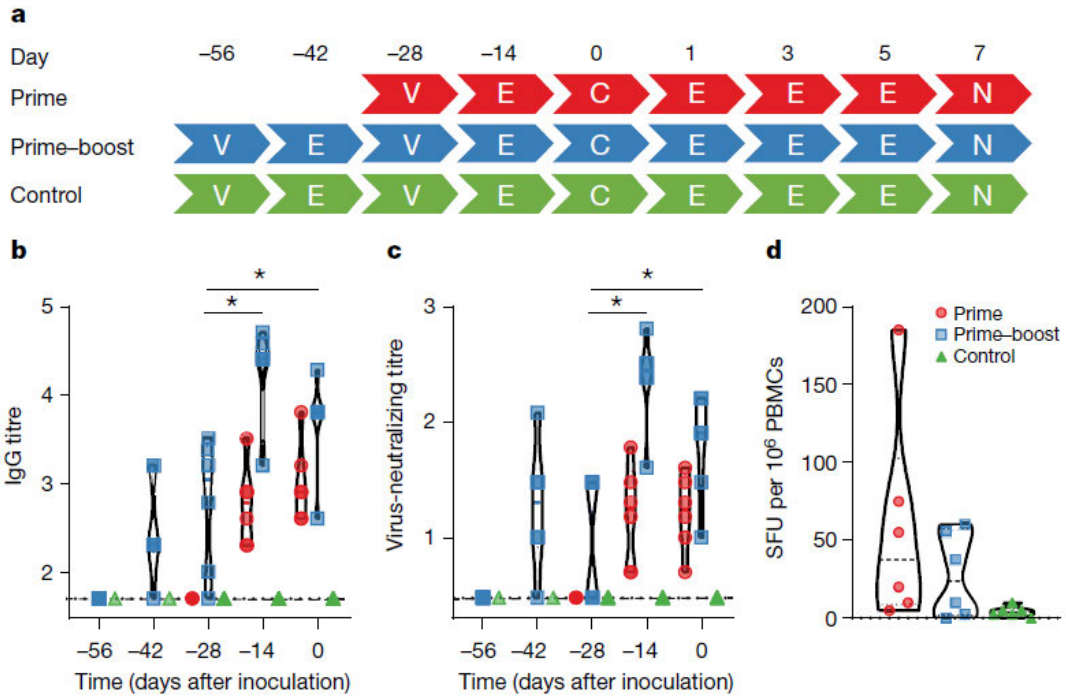
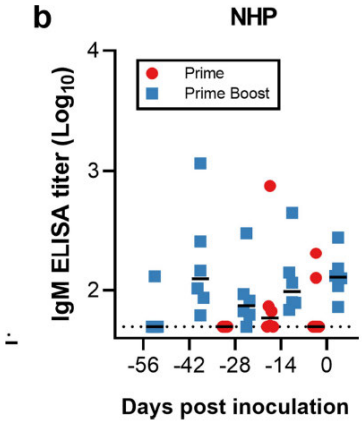


Figure 2. Humoral and cellular immune responses to ChAdOx1 nCoV-19 vaccination in rhesus macaques
a, Study schedule for NHPs. **C,** examination and challenge**; E,** examination**; N,** examination and necropsy**; V,** vaccination**.** **b,** Endpoint IgG titre in serum against trimeric spike protein. log_{10}-transformed IgG endpoint titrations are shown, which were obtained by ELISA. **c,** log_{10}-transformed virus-neutralizing titres in the serum. **d**, Summed IFNγ ELISpot responses in peripheral blood mononuclear cells collected on the day of challenge to peptides that span the full length of the spike protein. Red circles, prime-only vaccine; blue squares, prime–boost vaccine; green triangles, controls. The dotted line shows the limit of detection. *P = 0.0313. Statistical significance was determined using two-tailed signed-rank Wilcoxon tests.

- IgM antibodies were present in the serum after vaccination on the day of the challenge in six out of six prime–boost and two out of six prime-only animals (Extended Data Fig. 2b).

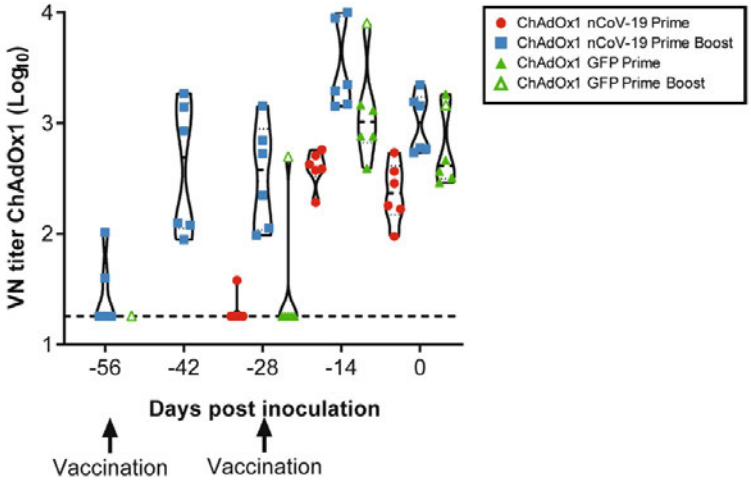
Extended Data Figure 2. Spike-specific serum IgM



Extended Data Figure 2. Spike-specific serum IgM

b, Spike-specific serum IgM in NHPs after prime–boost or prime-only vaccination. n = 6 animals per group examined in 2 independent experiments

- Vaccination with ChAdOx1 nCoV-19 resulted in the induction of NAbs against the vaccine vector itself within 28 days of vaccination (Extended Data Figure 3).
- An increase in the SARS-CoV-2 virus-NAb titre was not significantly correlated with the ChAdOx1 virus-neutralizing titre (two-tailed Pearson correlation, $r^2 = 0.6493$ P = 0.0529)



Extended Data Figure 3 | ChAdOx1 neutralizing antibodies in serum of vaccinated NHPs.

The control NHP treated with the prime–boost regimen is shown as an open triangle. VN, virus neutralizing. *n* = 6 animals per group examined in 2 independent experiments

Clinical signs post challenge

- After challenge in both the upper and lower respiratory tracts of rhesus macaques, the average clinical score of control animals was higher compared with ChAdOx1 nCoV-19-vaccinated animals. This was significantly different on 4–7 DPI (Figure 3a).

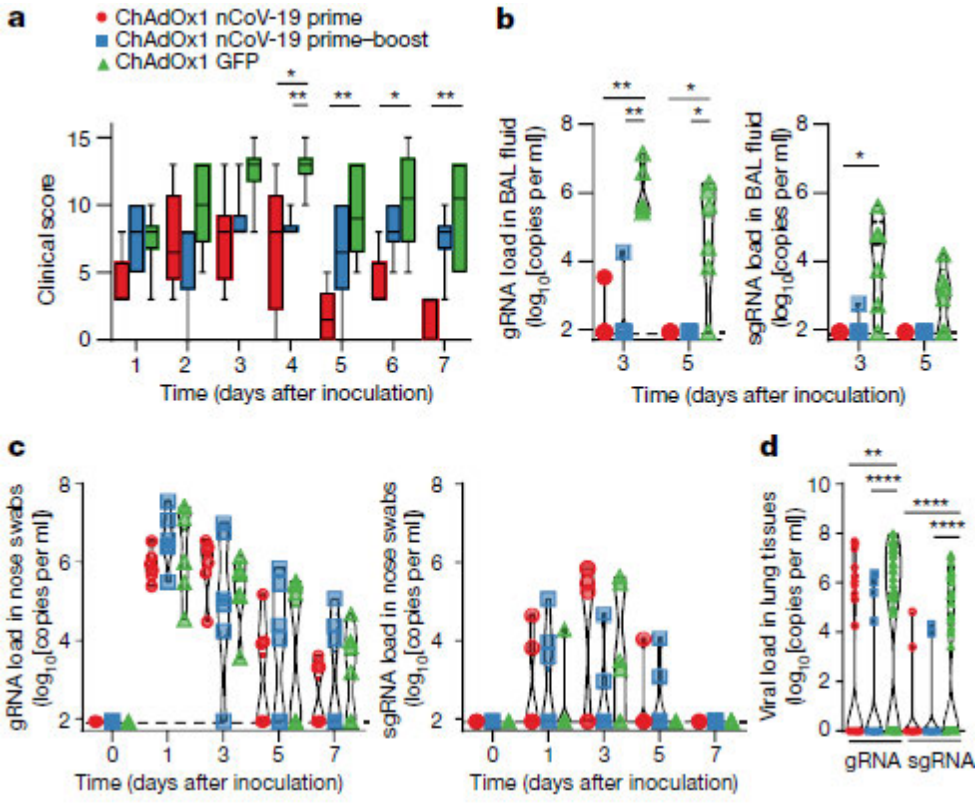


Figure 3 | Clinical signs and viral load in rhesus macaques inoculated with SARS-CoV-2 after vaccination with ChAdOx1 nCoV-19.

a, Clinical score in NHPs. Boxes show the 25th to 75th percentiles, the centre line is the median and whiskers range from the 5th to 95th percentiles. *n* = 6 animals per group were analysed in two independent experiments. P values for the indicated comparisons were as follows: P = 0.0238 (prime and control, 4 DPI.); P = 0.0043 (prime–boost and control, 4 DPI); P = 0.0043 (prime and control, 5 DPI); P = 0.0152 (prime and control, 6 DPI); P = 0.0022 (prime and control, 7 d.p.i.). **b, c,** Viral load in BAL fluid (b) (*P = 0.0152; **P = 0.0022) and nose swabs **(c) obtained from rhesus macaques. d,** Viral load in lung tissue. n = 6 lung lobes of six animals per group analysed in two independent experiments. **P = 0.0011; ****P < 0.0001. The dotted line indicates the limit of detection. Statistical significance was determined using two-tailed Mann–Whitney U-tests.

Viral Load in respiratory tract post challenge

- In BAL fluid obtained from control animals, viral genomic RNA (gRNA) and subgenomic RNA (sgRNA), the latter of which is indicative of virus replication, were detected on all days.
- Viral gRNA and sgRNA were detected in only two vaccinated animals on 3 DPI, and the viral load was significantly lower (Figure 3b).
- Viral gRNA was detected in nose swabs from all animals and no difference was found on any day between vaccinated and control animals.
- Viral sgRNA was detected in a minority of samples, with no difference between groups (Figure 3c).
- Infectious virus could only be detected at 1 and 3 DPI in prime-only vaccinated and control animals, and 1 DPI in prime–boost vaccinated animals (Extended Data Table 1) from nasal swabs.

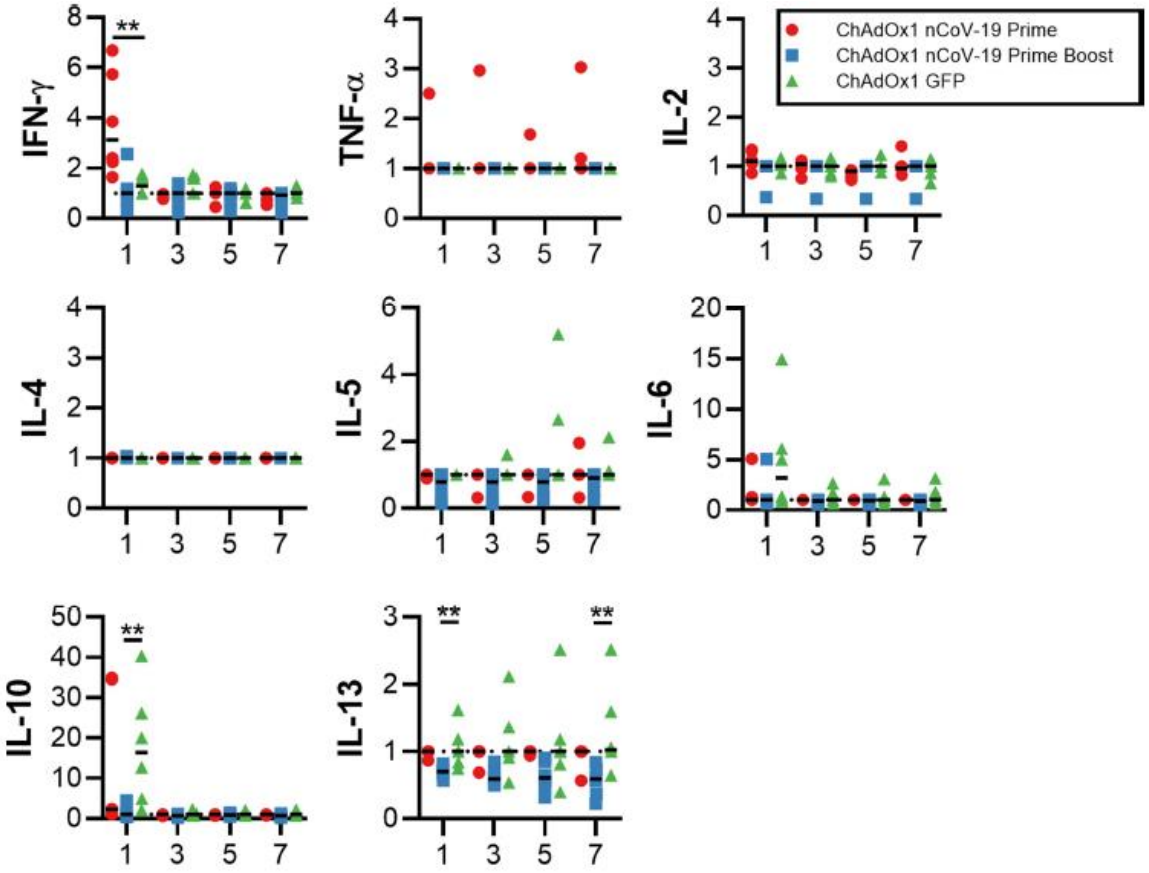
Extended Data Table 1 | Virus isolation from nasal swabs

	ChAdOx1 nCoV-19 Prime	ChAdOx1 nCoV-19 Prime-boost	ChAdOx1 GFP
1 DPI	4/6	2/6	4/6
3 DPI	2/6	0/6	1/6
5 DPI	0/6	0/6	0/6
7 DPI	0/6	0/6	0/6

Virus was isolated from nasal swabs of rhesus macaques after challenge with SARS-CoV-2.

Cytokine Response

- After prime-boost vaccination, the levels of TH1 (IFNγ and IL-2) or type-2 T helper cell (TH2) (IL-4, IL-5 or IL-13) cytokines in the serum of NHPs were low and no evidence of a dominant TH2 response was detected (Extended Data Fig. 4).
- Significant upregulation in IFNγ at 1 DPI in ChAdOx1 nCoV-19 prime-only-vaccinated animals, but not in prime–boost-vaccinated or control animals.
- IL-10 and IL-13 were significantly upregulated in control animals compared with prime–boost-vaccinated animals on 1 and 7 DPI (IL-13 only), but not compared with prime-only-vaccinated animals.
- No significant differences were observed between ChAdOx1 nCoV-19-treated and control animals for TNF, IL-2, IL-4, IL-5 and IL-6 (Extended Data Fig. 4)

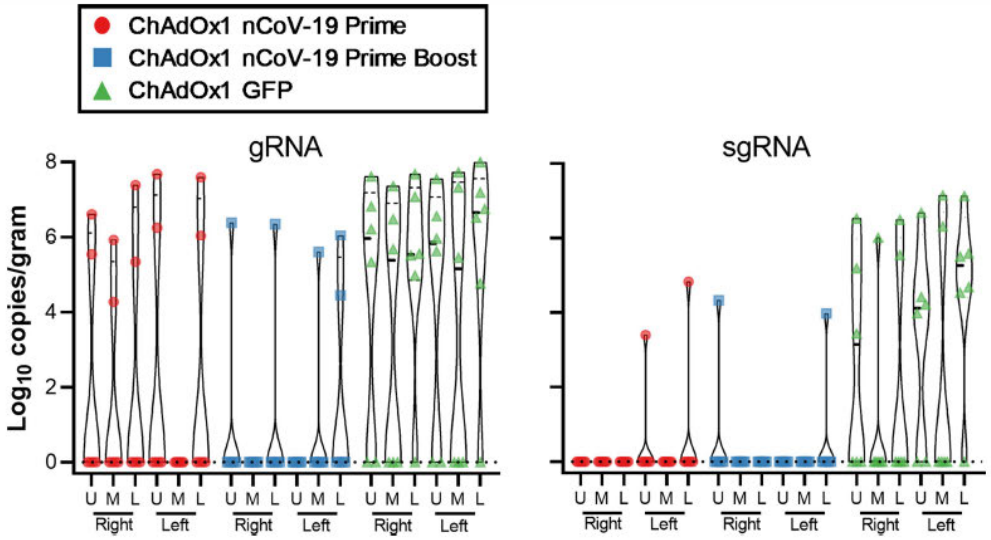


Extended Data Figure 4. Serum cytokines in rhesus macaques challenged with SARS-CoV-2.
Fold increase in cytokines in serum compared to pre-challenge values. **P < 0.01. The lines indicate the median. Statistical significance was determined by two-tailed Mann–Whitney U-test. P values for the different cytokines were: IFNγ, P = 0.0087; IL-10, P = 0.0043; IL-13, P = 0.0043 (left) and P = 0.0065 (right). *n* = 6 animals per group examined in 2 independent experiments.

Pulmonary pathology and viral load

- None of the vaccinated monkeys developed pulmonary pathology after inoculation with SARS-CoV-2. All lungs were histologically normal and no evidence of viral pneumonia nor immune-enhanced inflammatory disease was observed.
- No SARS-CoV-2 antigen was detected by immunohistochemistry in the lungs of any of the vaccinated animals.
- Three out of six control animals developed some degree of viral interstitial pneumonia. Lesions were widely separated and characterized by thickening of alveolar septae by small amounts of oedema fluid and few macrophages and lymphocytes. Alveoli contained small numbers of pulmonary macrophages and, rarely, oedema. Type-II pneumocyte hyperplasia was observed. Multifocal, perivascular infiltrates of small numbers of lymphocytes that formed perivascular cuffs were observed. Immunohistochemistry analysis showed the presence of viral antigen in type-I and II pneumocytes, as well as in alveolar macrophages, in five out of six control animals.
- No lesions or immunohistochemistry-positive cells in nasal mucosa in any of the animals.
- The viral gRNA load was high in the lung tissues of all control animals and viral sgRNA was detected in five out of six control animals (Figure 3d).
- In the prime-only-vaccinated group, the viral gRNA load was significantly lower in lung tissues and below the limit of detection in two out of six vaccinated animals; viral sgRNA was detected only in the lung tissue of one animal (Figure 3d and Extended Data Figure 5).

- In the prime–boost-vaccinated group, viral gRNA was detected in two out of six animals; one animal was weakly positive in one lung lobe and one animal, which mounted a limited response to vaccination, was positive in four lung lobes.
- Viral sgRNA could only be detected in lung tissue from the animal with a lower immune response (Figure 3d and Extended Data Figure 5).
- No infectious virus was detected in any lung tissue.

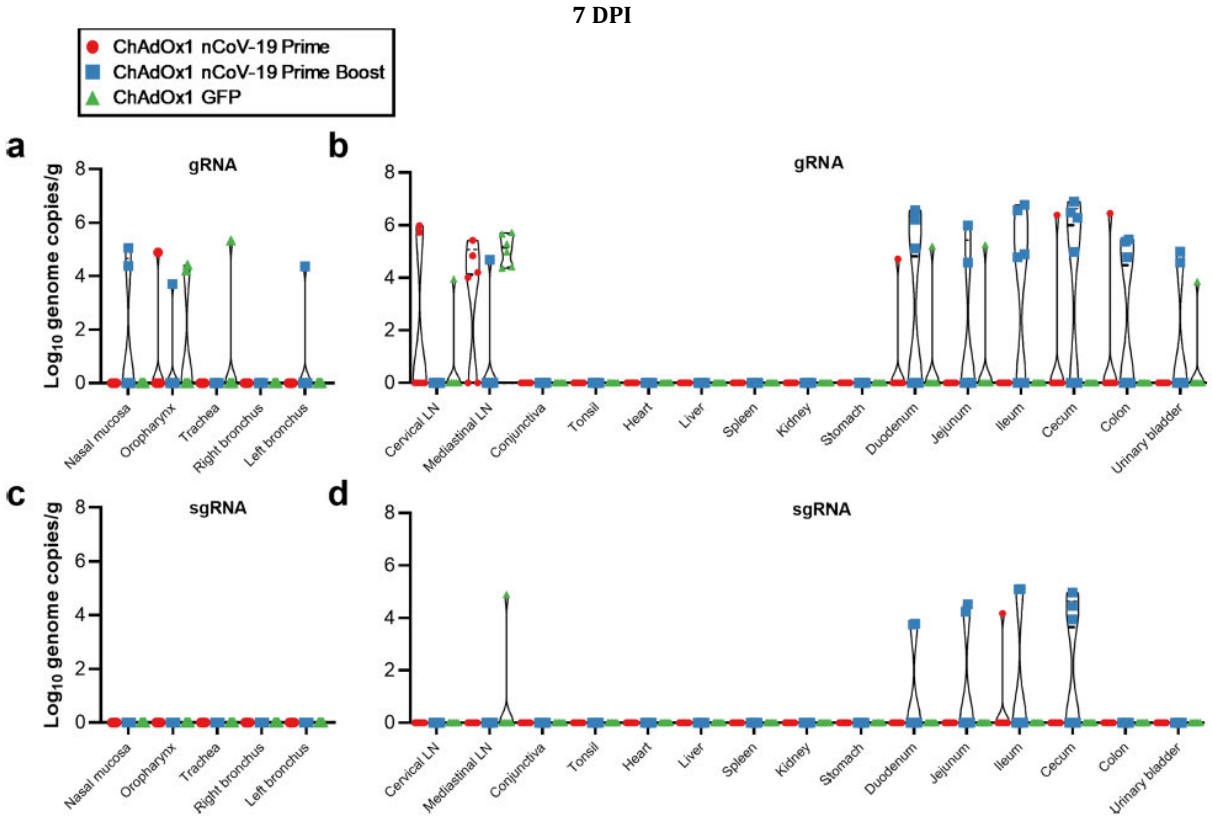


Viral gRNA (left) and sgRNA (right) in lung tissues.

Extended Data Figure 5. Viral load in lung tissue of rhesus macaques challenged with SARS-CoV-2 at 7 DPI

Extrapulmonary pathology and viral load

- No lesions were observed in gastrointestinal tissues of any animals.
- SARS-CoV-2 antigen could be detected in lymphocytes and macrophages in the lamina propria of the intestinal tract of all control animals. This was also observed in six out of six prime-only-vaccinated animals and three out of six prime–boost-vaccinated animals.
- There were no histological differences between lymphoid tissues of vaccinated or control animals.
- Viral gRNA could be detected in extrarespiratory tissues but was predominantly low in all animals and not associated with the detection of sgRNA.
- The viral gRNA load in intestinal tissues of prime–boost-vaccinated animals was higher than the levels measured in control and prime-only-vaccinated animals and was associated with the detection of sgRNA.
- No infection of intestinal tissue was observed by immunohistochemistry, no infectious virus was detected in intestinal tissue (Extended Data Figure 6).



Extended Data Figure 6. Viral load in tissues of rhesus macaques challenged with SARS-CoV-2 at 7 DPI. a, Viral gRNA in respiratory tissues excluding lung tissue. **b,** Viral gRNA in non-respiratory tissues. **c,** Viral sgRNA in respiratory tissues excluding lung tissue. **d,** Viral sgRNA in non-respiratory tissues.

Conclusion:

Prime-boost showed a higher response to IgG (IgG subtypes not provided) and NAb than a single dose but increase in titres only lasted for 14 days. ELISpot responses were at least 3-fold higher in prime only animals compared to prime-boost animals but was reported to be not statistically significant. Prime-boost vaccination before challenge had limited benefit in improving clinical scores up to 7 DPC compared to single dose vaccination. BAL gRNA and sgRNA levels reported were similar between prime-only and prime-boost vaccinated animals compared to high levels seen in controls, so no relevant benefit could be concluded between prime and prime-boost animals in this set of data. In lung tissues there appeared to be a decrease in RNA (both gRNA and sgRNA) compared to controls, however due to high degree of variability among animals these findings are not conclusive. An unexplained increase in viral RNA was observed in the gastrointestinal tract at 7DPC generally in prime-boost immunised animals but not prime only or controls and requires further investigation. Cytokine measurements following challenge revealed IFN-γ levels in prime-only animals on 1DPC, but not in prime-boost animals at the same timepoint. Vaccine related cytokine measurement pre-challenge was not determined. Overall the vaccine provided some protection in vaccinated animals compared to controls. Deficiencies included absence of cellular immune response during vaccination phase and long-term immunity/protection studies.

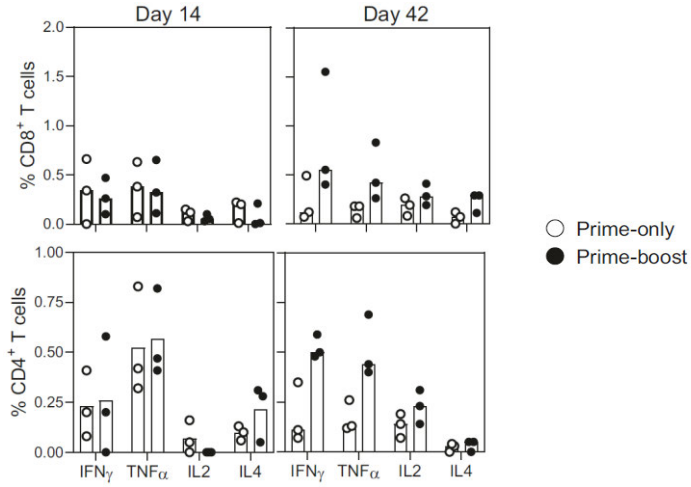
PORCINE IMMUNOGENICITY

Report no. AR001111, & Graham et al. Vaccines. 2020;5:69 https://doi.org/10.1038/s41541-020-00221-3

Female Large White-Landrace-Hampshire cross-bred pigs (n=3/ group)
Age: 8-10 weeks
Immunisation: Day 0 (prime only) and 28 (prime and boost)
Route and dose: IM, 5.12×10^{10} vp AZD1222

[redacted]

- Both CD4+ and CD8+ T cell responses (by ICS analysis) were induced. There are no day 0 baseline data. The Th1 response (expression of IFN-γ and TNF-α) of both CD8+ and CD4+ T cells was higher than the Th2 response (IL-4).



SARS-CoV-2 –specific T cell responses from ChAdOx1 nCoV-19 prime-only and prime-boost vaccination regimens in pigs. Each data represents an individual pig with bars denoting the median response per group/time point.

Conclusions: Study results show a trend towards higher humoral (NAbs) and cellular responses (IFN–γ, CD4+, CD8+ T cells) in animals vaccinated with the prime-boost regimen. Addition of a booster dose of the vaccine significantly enhanced the immune response, at least for a short period of time measured in the study (14 days). The T cell findings suggest a Th-1 biased immune response.

AZD1222 ANTIBODIES AGAINST THE D614G MUTANT

[redacted]

[redacted]

SAFETY PHARMACOLOGY

[redacted]

PHARMACOKINETICS

DISTRIBUTION & SHEDDING

[redacted]

Distribution and shedding study of ChAdOx1-HBV

Study no.: 0841MV38.001 (GLP compliant)

Vaccine: ChAdOx1-HBV (MVA-HBV was not discussed)
Species: Balb/C mice
Dose/route: IM as outlined in Table. Group 2 was only administered a single dose, Group 3 & 4 were administered 2 doses 28 days apart and group 1 (control) were administered saline prior to day 1

Group	# of animals/sex	ChAdOx-1 HBV (VP/dose)	Day 1	Day 28	Euthanised
1	2 ♂/2 ♀	0	-	-	Day 29
2	5 ♂/5 ♀	2.4×10^{10}	ChAdOx1-HBV administered by IM route	-	Day 1
3	4♂/5 ♀			ChAdOx1-HBV administered by IM route	Day 29
4	6♂/5♀				Day 56

Tissue collection: Group 2 - 24 h after dosing on Day 1, Groups 1 and 3 - 24 h after the second (boost) dosing on day 28 & Group 4 on Day 56, 28 days after the second (boost) dose.
Biodistribution samples: whole blood, injection site (skeletal muscle), brain, heart, draining inguinal lymph node, kidney, liver, lung, gonads, and spleen.
Shedding: urine, faeces (up to day 35)
Bioanalytical: qPCR - QuantStudio 7 Flex Real-Time PCR System detecting ChAdOx1 – backbone.
The detection limit was Ct value <40 (~30 DNA copy numbers). The precision of each standard level was as follows: the %CV of the calculated DNA copy number for the remaining wells of each standard level was ≤ 15%, except for the LOQ and 10X LOQ, which was ≤ 35% and r^2 was ≥0.96.

Results:
Distribution: ChAdOx1-HBV was not present in whole blood samples at any time-point. Distribution to all tissues was noted on day 2 and day 29 and this included brain, heart, kidney, liver, lung, lymph node, skeletal muscle, spleen, testes and ovaries. The highest levels (copies/mg sample) were noted in skeletal muscle at the injection site, with the copy number noted on day 2 (1 day after the first administration) ranging from 3×10^5 to 10×10^6 copies/µg sample. Due to experimental error, comparison of copy number in skeletal muscle on Day 29 and Day 56 was not determined. On day 56 only low levels were noted in 1 sample (of 7) for brain, heart and liver, 1 of 3 for ovary, 1 of 4 for testes and 4 of 7 in lymph node samples at this timepoint (see Table below). Note whole blood was negative at all time points.

Mean copy numbers/µg sample in positive tissues

Tissue	Day 1	Day 29	Day 56
Brain	454 (6/10 pos)	2.8E+3 (4/8 pos)	107 (1/7 pos)
Heart	366 (3/10 pos)	9.8E+3 (1/8 pos)	50 (1/7 pos)
Kidney	219 (3/10 pos)	47 (1/8 pos)	0 (0/7pos)
Liver	378 (8/10 pos)	106 (3/8 pos)	126 (1/7 pos)
Lung	229 (8/10 pos)	66 (2/8 pos)	0 (0/7 pos)
Draining Lymph Node	2.8E+4 (8/10 pos)	1.14E+6 (7/8 pos)	1.3E+3 (4/7 pos)
Ovary	125 (1/5 pos)	181 (3/5 pos)	498 (1/3 pos)
Skeletal Muscle	9.75E+6 (10/10 pos)	Experimental error	
Spleen	1.43 E+3 (9/10 pos)	9.09E+6 (4/8 pos)	0(0/7 pos)
Testis	130 (2/5 pos)	0 (0/3 pos)	34 (1/4 pos)

Shedding: Faecal (41 samples) and urine (39 samples) showed no shedding in these matrices at the time points sampled.

Distribution studies with AdCh63 in mice

<table>
<tr><th>Study details</th><th>Major Findings</th></tr>
<tr><td>Study: UNO0014/RMPBioDIST-001
Vaccine: AdCh63 MSP-1 and MVA MSP-1 (chimpanzee adenovirus 63-vectored malaria vaccine and modified vaccinia Ankara–vectored malaria vaccine targeting MSP-1)
Species: Balb/C mice
Dose/route: 1 × 10¹⁰ VP, AdCh63 MSP-1 and 1.037x10⁸pfu, MVA MSP-1/ IM (hindlimb)
Harvest: days 0, 1 and 8
Tissue/organs investigated: injection site, draining lymph nodes (DLNs), liver, spleen, gonads (either ovaries or testes and epididymis).
Virus detection: tissues homogenised, 3 freeze-thaw cycles, centrifuged, infected permissive cells. Permissive cells (no details provided) were exposed to the clarified samples for 4 days, virus harvested inoculated to cell monolayer and subsequently immunostained using either anti-vaccinia antibody (for MVA) or anti-hexon antibody (for AdCh63).
GLP</td><td><u>AdCh63 MSP-1</u>
<table><tr><th></th><th colspan="3">Percentage positive</th></tr><tr><th></th><th>Immediate 1/sex</th><th>24 h 3/sex</th><th>Day 8 3/sex</th></tr><tr><td>Injection site</td><td>100%</td><td>0%</td><td>0%</td></tr><tr><td>DLN</td><td></td><td>0%</td><td>0%</td></tr><tr><td>Spleen, Gonads & liver</td><td></td><td>0%</td><td>0%</td></tr></table>
<u>MVA MSP-1</u>
<table><tr><th></th><th colspan="3">Percentage positive</th></tr><tr><th></th><th>Immediate 1/sex</th><th>24 h 3/sex</th><th>Day 8 3/sex</th></tr><tr><td>Injection site</td><td>100%</td><td>100%</td><td>100%</td></tr><tr><td>DLN</td><td></td><td>66.6%</td><td>0%</td></tr><tr><td>Spleen, Gonads & liver</td><td></td><td>0%</td><td>0%</td></tr></table>
Comment: Detection system not sensitive. Need more sensitive method such as PCR</td></tr>
<tr><td>Study: UNO0009/MAB-001
Vaccine: AdCh63ME-TRAP
Species: Balb/C mice
Dose/route: 3.3 x 10⁹ VP/ 15 µl/ear
Terminated: Day 8
Tissue/organs investigated: ovaries, testes and epididymis (pooled), cervical lymph nodes, ovary, liver and spleen
Virus detection: grinding mouse tissue samples and inoculating HEK293 cells, 5 days incubation followed by Real Time-PCR to detect viral growth (no optimisation of PCR/or cycle threshold (CT) data provided).
GLP</td><td>• No infectious AdCh63 ME-TRAP virus particles were detected in any internal organ (reproductive organs, spleen, liver, cervical lymph nodes).
• One week after intradermal injection the AdCh63 ME-TRAP was only detected at the injection site (dermis on the rear aspect of each pinna).
• There was no evidence of replication of the virus or presence of a disseminated infection

Comment: Ear not a relevant route. Only a summary report.</td></tr>
</table>

SINGLE-DOSE TOXICITY

No single dose toxicity studies have been performed with AZD1222.

REPEAT-DOSE TOXICITY

A repeat dose GLP study with AZD1222 and two toxicology studies with the same replication incompetent chimpanzee adenoviral vectored vaccines were provided . A human adenovirus vector AdCh63 vector ([redacted]) was also provided, but has not been reviewed, as this study is not considered relevant.

Table 0.1. Overview of repeat-dose toxicity studies

Species & strain	Study	Vaccine	Recovery	Route	Dose (VP); frequency		Date	GLP
Mouse (Balb/C)	513351	AZD1222	28 days	IM	3.7×10^{10}	Days 1, 22 and 43	30/12/20	Yes
Mouse (Balb/C)	QS18DL	ChAdOx1 MERS & ChAdOx1 Chik	13 days	IM	1×10^{10}	Days 1 &15	1/12/18	Yes
Mouse (Balb/C)	XMM0003	ChAdOX1 NP+M1 (Day 1)	13 days	IM	1×10^{10} VP + 1.5×10^{7} pfu	Days 1 and 15	26/02/14	Yes

6-week study with AZD1222

[redacted]

Major findings

Mortality: No test related mortality

Clinical signs: No test related effect

Dermal scoring: No clinical observations of erythema/oedema at injection site

Body weight gain: No test related effect

Body temperature: ↑ in ♂ on Day 1 & statistically significant ↑ Day 22, 4 h postdose (mean of 38.25°C c.f. 36.98°C controls)

Irwin observations: no effects on body temperature, pupil size or Irwin observations, when assessed in ♂ on day 8 & 29

Haematology: ↓ monocytes ♂ (0.43x) & ♀ (0.39x; statistically significant) on Day 45

Serum chemistry:

AZD1222- related plasma chemistry

Dose	0		3.7×10^{10}	
Day 45 (n=10)				
Globulin#	17.90	15.26	1.19***	1.22**
Albumin#	31.29	34.85	0.94*	0.90**
Day 74 (recovery period)				
Globulin#	-	12.95	-	1.19*

 # concentration in g/L for the control groups and fold **change** for the treated **group**s relative to the respective control group; * p < 0.05; ** p < 0.01; *** p < 0.001.

Organ weights (bodyweight-relative): statistically significant ↑ in spleen weight by 1.3x ♂ & 1.6x ♀ and normal end of recovery

Gross pathology: no AZD1222 related findings

Histopathology:

Sex	♂		♀	
Viral Particle (VP)/dose	0	3.7×10^{10}	0	3.7×10^{10}
Number of animals	10	10	10	10
Administration site 1 (*n* =10)				
Inflammation, subcutaneous tissue; myofiber, minimal	2	10	1	10
Mononuclear cell	2	6	0	3
Mixed cell	0	4	1	7
Oedema, subcutaneous, minimal	8	9	7	5
Haemorrhage, subcutaneous, minimal	0	0	0	1
Administration site 2 (*n* =10)				
Inflammation, subcutaneous tissue; myofiber, minimal	4	10	1	9
Mononuclear cell	4	6	0	1
Mixed cell	0	4	1	8
Oedema, subcutaneous, minimal	9	9	10	4
Haemorrhage, subcutaneous, minimal	2	2	0	2
Congestion, subcutaneous, minimal	1	0	0	0
Sciatic Nerve (*n*=10)				
Inflammation, perineurial, minimal	0	9	2	9
Mononuclear cell	0	5	0	1
Mixed cell	0	4	2	8

Inflammatory cells at the sciatic nerve were epineurial/perineurial and did not extend into the endoneurium. No findings were present in the underlying axons. Inflammation of at the sciatic nerve resulted from an extension of inflammation from the adjacent injection site.

Recovery animal: no histopathology findings considered related to the administration of AZD1222

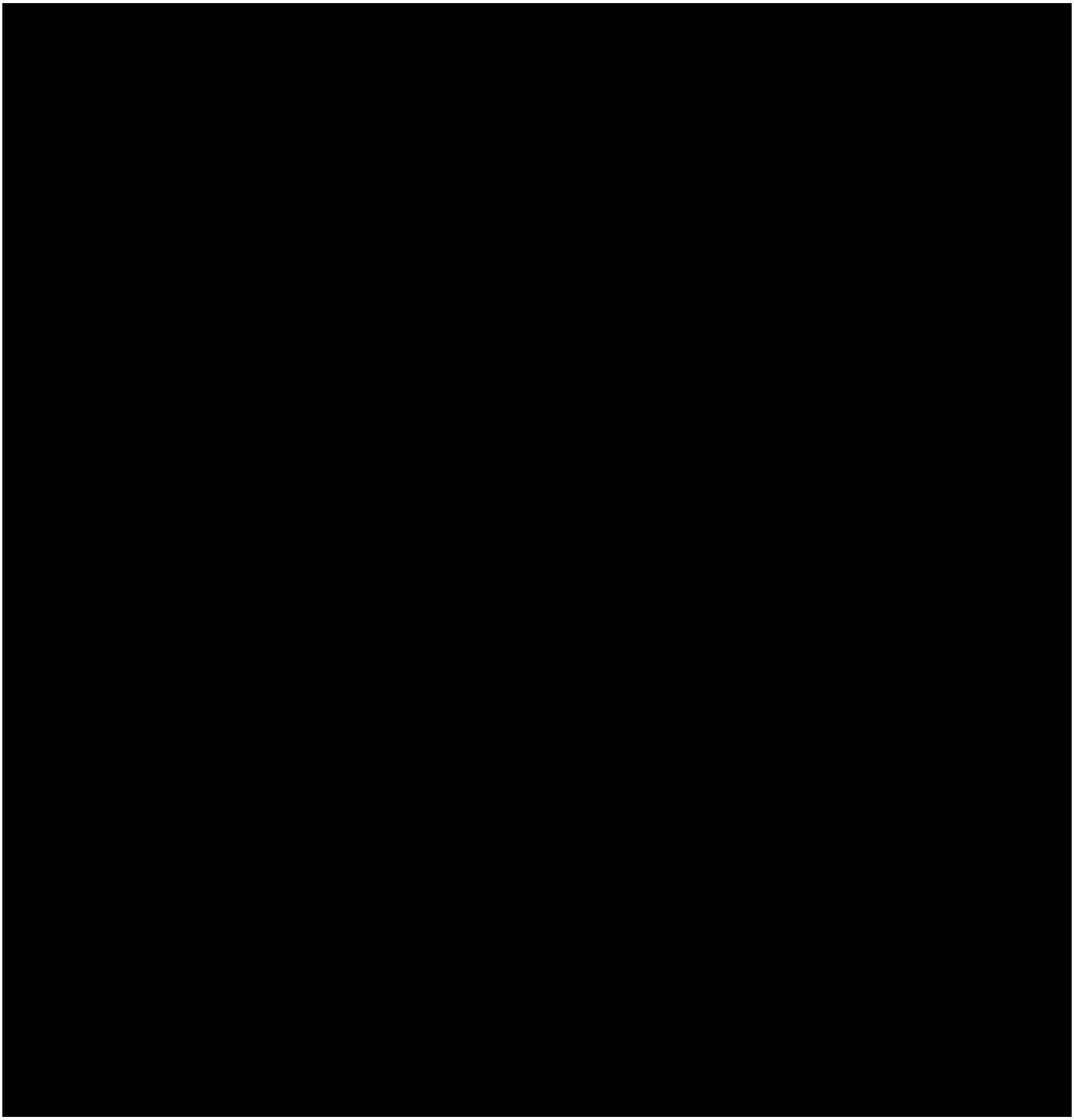
Immunogenicity results:

Mean immunogenicity Response: S-specific Antibody Concentration (AU/mL)

	♂	♀
Day 22	372.405	816.330
Day 43	993.835	1636.206
Day 74 (recovery period)	1240.280	2477.321

All samples collected from **animals during the pretreatment phase prior to immunisation were below the limit of quantification (BLQ) for the assay (0.250 AU/mL) and considered seronegative. The S antibody was measured by an ECL immunoassay using SULFO-TAG labelled goat anti-mouse antibody and plates coated with recombinant SARS-CoV-2 trimeric spike protein.**

NOAEL: not determined



LOCAL TOLERANCE

The local effects observed in the toxicity study with the ChAdOx vector vaccines (see repeat dose studies) are considered non adverse and are generally related to the inflammatory reaction to the vaccine. No dedicated local tolerance studies was provided for AZD1222

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