ChAdOx1 nCoV-19 (AZD1222) protects hamsters against SARS-CoV-2 B.1.351 and B.1.1.7 disease

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Abstract

We investigated ChAdOx1 nCoV-19 (AZD1222) vaccine efficacy against SARS-CoV-2 variants of concern (VOCs) B.1.1.7 and B.1.351 in Syrian hamsters. We previously showed protection against SARS-CoV-2 disease and pneumonia in hamsters vaccinated with a single dose of ChAdOx1 nCoV-19. Here, we observed a 9.5-fold reduction of virus neutralizing antibody titer in vaccinated hamster sera against B.1.351 compared to B.1.1.7. Vaccinated hamsters challenged with B.1.1.7 or B.1.351 did not lose weight compared to control animals. In contrast to control animals, the lungs of vaccinated animals did not show any gross lesions. Minimal to no viral subgenomic RNA (sgRNA) and no infectious virus was detected in lungs of vaccinated animals. Histopathological evaluation showed extensive pulmonary pathology caused by B.1.1.7 or B.1.351 replication in the control animals, but none in the vaccinated animals. These data demonstrate the effectiveness of the ChAdOx1 nCoV-19 vaccine against clinical disease caused by B.1.1.7 or B.1.351 VOCs.

Main

The COVID-19 pandemic produced an unprecedented development of SARS-CoV-2 vaccines, and just over a year after the beginning of the outbreak a total of 12 vaccines have been authorized or approved globally. As the pandemic progressed, several VOCs have been detected. These include the B.1.1.7 and B.1.351 VOCs. The B.1.1.7 VOC was first detected in the United Kingdom and has seven amino acid (AA) substitutions and two deletions in the spike protein[1,2] compared to the original Wuhan isolate, Wuhan-Hu-1. The B.1.351 VOC was first detected in South Africa and has eight AA substitutions and one deletion in the spike protein[3] (Table 1). All currently licensed vaccines are based on the spike protein of Wuhan-Hu-1, thus, concerns have

43 been raised that the presence of these changes may affect vaccine efficacy. The goal of this study
44 was to evaluate ChAdOx1 nCoV-19 (AZD1222) vaccine efficacy in Syrian hamsters, when
45 challenged using naturally occurring isolates of the VOCs B.1.1.7 and B.1.351.
46

Substitution (Wuhan AA numbering)	**VOC B.1.1.7**[1]	**VOC B.1.351**[3]
L18F	-	+
HV69-70del	+	-
D80A	-	+
Y144del	+	-
D215G	-	+
LAL242-244del	-	+
K417N	-	+
E484K	-	+
N501Y	+	+
A570D	+	-
D614G	+	+
P681D	+	-
A701V	-	+
T716I	+	-
S982A	+	-
D1118H	+	-

47 Table 1. AA substitutions detected in the spike protein of VOCs B.1.1.7 (EPI_ISL_601443) and
48 B.1.351 (EPI_ISL_678615) compared to Wuhan-Hu-1 (NC_045512).
49

50 Syrian hamsters (N=10 per group) were vaccinated intramuscularly with either ChAdOx1 nCoV-
51 19 or ChAdOx1 green fluorescent protein (GFP, 2.5 x 10^8 IU/hamster) 30 days prior to intranasal
52 challenge with SARS-CoV-2 VOCs B.1.1.7 or B.1.351. Vaccination with ChAdOx1 nCoV19
53 resulted in high titers of binding antibodies against the SARS-CoV-2 full-length spike protein
54 and receptor binding domain (Figure 1a) at 25 days post vaccination. We then investigated
55 neutralizing antibody titers in serum against infectious virus. Neutralization of B.1.351 was
56 significantly reduced compared to neutralization of B.1.1.7 (Figure 1b, mean titer of 15 vs 142, p
57 < 0.0001, Mann-Whitney test).

58 Virus stocks were deep sequenced before inoculation. No mutations were found in B.1.1.7
59 compared to the isolate's sequence, but two AA substitutions were found in the spike protein of
60 B.1.351; Q677H (present at 88%) and R682W (present at 89%).
61 Upon inoculation with virus, weight loss was observed in control hamsters challenged with
62 B.1.1.7, whereas less pronounced weight loss was observed in control hamsters challenged with
63 B.1.351 (Figure 1c). In contrast, vaccinated hamsters continued to gain weight throughout the
64 experiment. A significant difference in weight between vaccinated and control hamsters was
65 observed starting at 4 days post infection (DPI) for B.1.1.7 and at 6 DPI for B.1.351 (Figure 1c,
66 Student's t-test corrected for multiple comparisons using the Holm-Šidák method) and continued
67 throughout the remainder of the experiment. Four out of ten hamsters per group were euthanized
68 at 5 DPI and lung tissue was harvested. Lung tissue of all control animals contained high levels
69 of sgRNA (Figure 1d, 10^8-10^{10} copies/gram tissue), and was comparable to sgRNA levels
70 previously detected in lung tissue of control animals challenged with SARS-CoV-2 D614G
71 (hCoV-19/USA/MT-RML-7/2020)[4]. Conversely, no sgRNA was detected in lung tissue obtained
72 from vaccinated hamsters challenged with B.1.1.7, and only one out of four vaccinated hamsters
73 challenged with B.1.351 had detectable sgRNA (Figure 1d, p=0.0286, Mann-Whitney test). High
74 levels of infectious virus were detected in lung tissue of all eight control animals, whereas no
75 vaccinated animals had detectable infectious virus in lung tissue (Figure 1e, p=0.0286, Mann-
76 Whitney test). Lung:body weight ratios on 5 DPI were significantly lower in vaccinated animals
77 compared to control animals for both VOCs (Figure 1f, p=0.0286, Mann-Whitney test),
78 indicating no or reduced pulmonary edema in ChAdOx1 nCoV19-vaccinated animals.
79

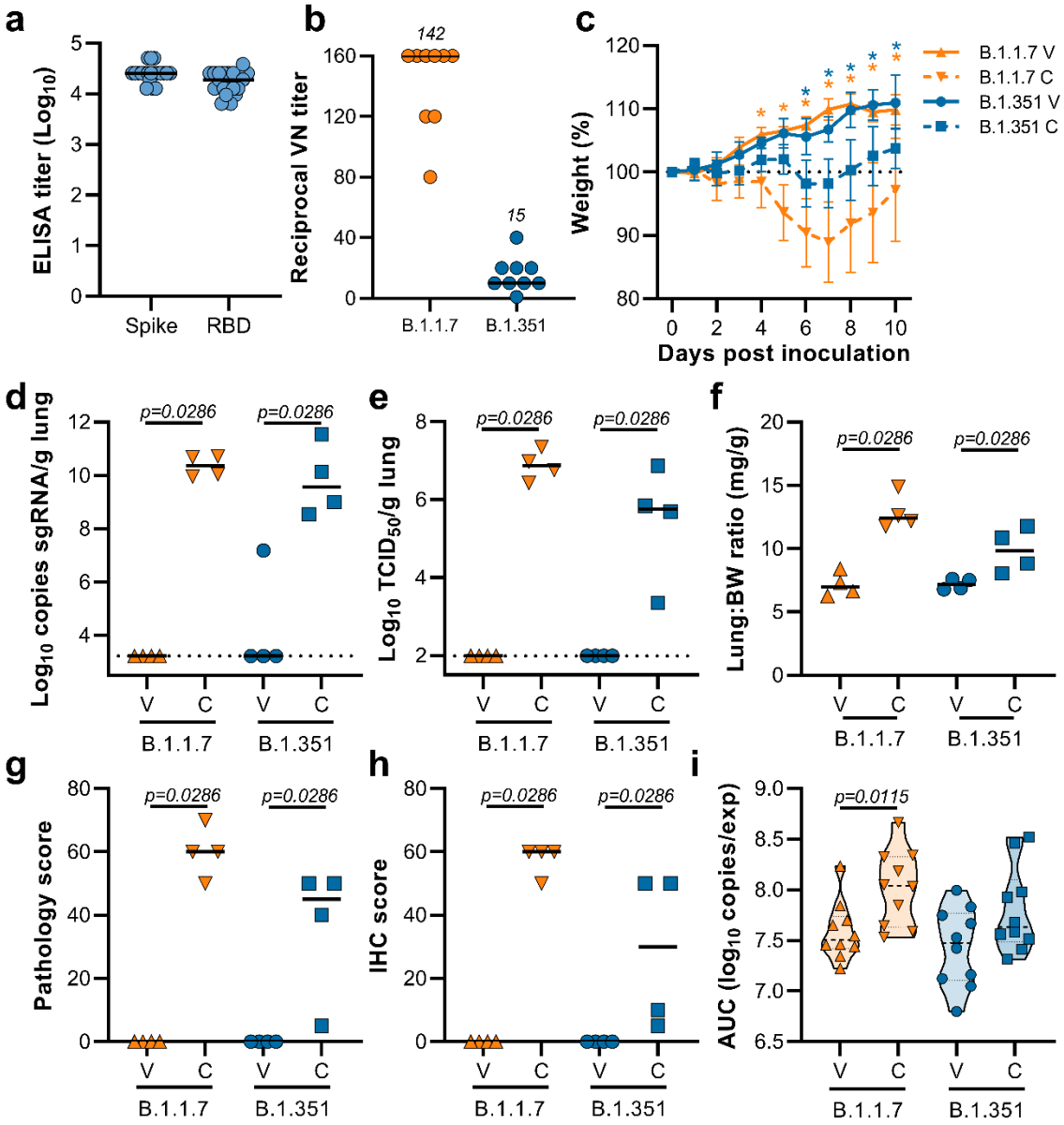
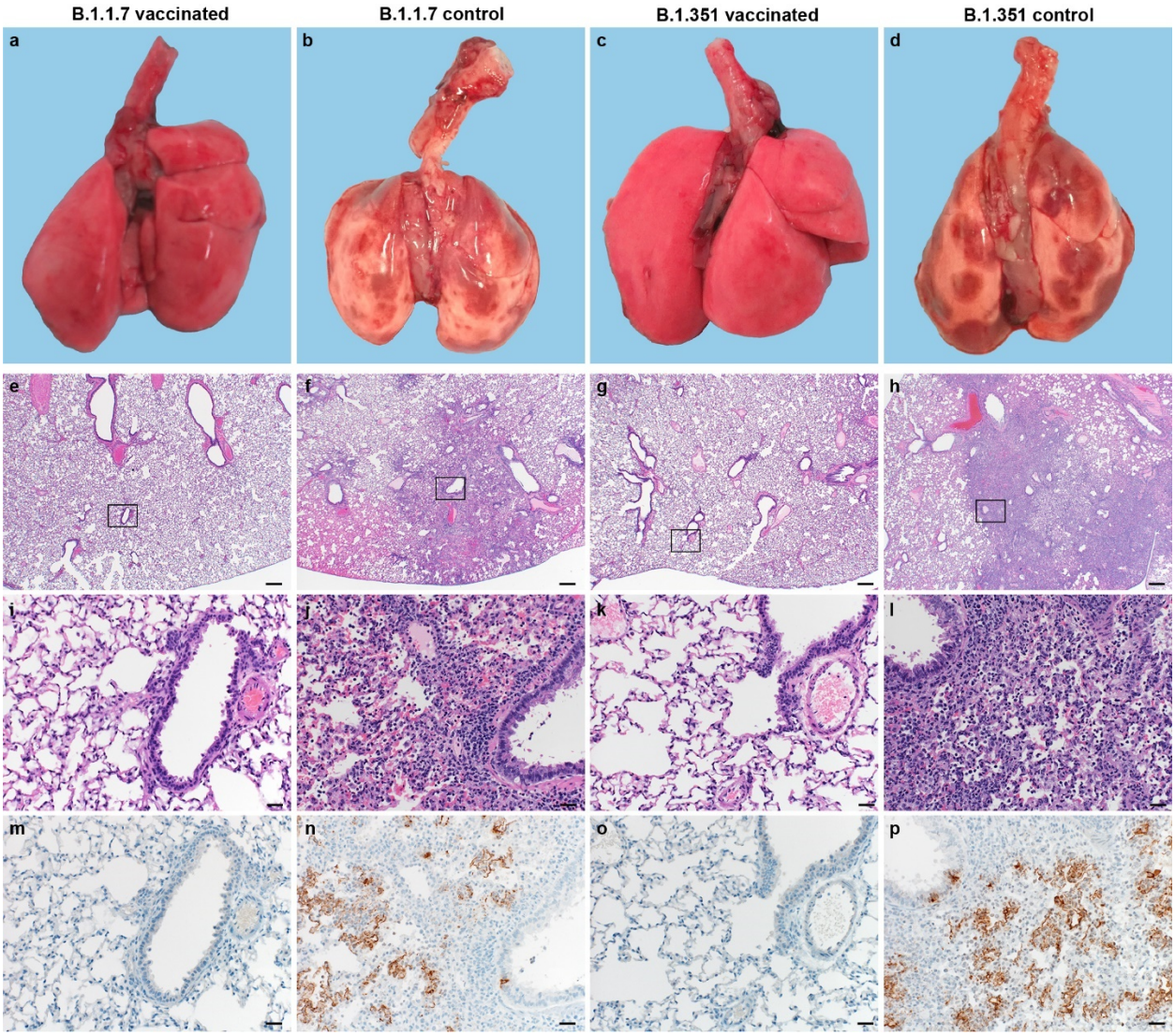


Figure 1. Vaccination of Syrian hamsters with ChAdOx1 nCoV-19 prevents lower respiratory tract infection with SARS-CoV-2 VOCs B.1.1.7 and B.1.351. a. Binding antibodies against spike protein or RBD of SARS-CoV-2 (clade A) in serum obtained 25 days post vaccination with ChAdOx1 nCoV-19. Line = median; numbers = mean. b. Virus neutralizing antibody titers against B.1.1.7 or B.1.351 VOCs in serum obtained 25 days post vaccination with ChAdOx1 nCoV-19. Line = median. c. Relative weight upon intranasal challenge with 10^4 $TCID_{50}$ of B.1.1.7 or B.1.351. Shown is geometric mean with 95% confidence interval (CI). * = p-value<0.005, corrected for multiple comparisons using the Holm-Šidák correction. d. sgRNA viral load in lung tissue obtained at 5 DPI. Line = median. Dotted

line = limit of detection. e. Infectious SARS-CoV-2 titer in lung tissue obtained at 5 DPI. Line = median. Dotted line = limit of detection. f. Lung:body weight (BW) ratio (mg:g) of hamsters euthanized at 5 DPI. Line = median. g. Percentage affected lung tissue per animal as determined via histology. Line = median. h. Percentage of lung tissue positive for SARS-CoV-2 antigen per animal. Line = median. i. Truncated violin-plot of area under the curve (AUC) analysis of shedding as measured by sgRNA analysis in swabs. Dashed line = median. Dotted line = quartiles. Statistical significance determined via Kruskall-Wallis test (b), mixed-effect analyses (c), or Mann-Whitney test (d-i). V = ChAdOx1 nCoV-19 vaccinated; C = ChAdOx1 GFP vaccinated; Orange upward triangle = Hamsters vaccinated with ChAdOx1 nCoV-19, challenged with B.1.1.7; Orange downward triangle = Hamsters vaccinated with ChAdOx1 GFP, challenged with B.1.1.7; Blue circle = Hamsters vaccinated with ChAdOx1 nCoV-19, challenged with B.1.351; Blue square = Hamsters vaccinated with ChAdOx1 GFP, challenged with B.1.351.

Upon necropsy, lungs from control animals showed gross lesions previously observed in hamsters inoculated with SARS-CoV-2 WA1 or a D614G isolate, with focal areas of hilar consolidation and hyperemia[4,5]. No gross lesions were observed in lung tissue obtained from any of the vaccinated animals (Figure 2a-d). Microscopically, pulmonary lesions of control animals consisted of a moderate to marked broncho-interstitial pneumonia extending into the adjacent alveoli. Bronchi and bronchioles had multifocal necrotic epithelial cells and moderate numbers of infiltrating neutrophils and macrophages. Alveolar septa were expanded by edema fluid and leucocytes. In contrast, vaccinated animals did not show any evidence of SARS-CoV-2 pathology (Figure 2e-l). Immunohistochemistry using a monoclonal antibody against SARS-CoV-2 demonstrated viral antigen in bronchial and bronchiolar epithelium, type I and II pneumocytes as well as pulmonary macrophages within the control animals, but not in vaccinated animals (Figure 2m-p). For the sections of lung evaluated, the percentage of lung tissue showing pathology and the percentage of lung tissue positive for SARS-CoV-2 antigen was determined by a veterinary pathologist blinded to the study group allocations. No pathology nor SARS-CoV-2 antigen was found in lungs of vaccinated animals, but both were abundantly present in lungs of control animals (Figure 1g,h, p=0.0286, Mann-Whitney test). Finally,

119 oropharyngeal swabs were collected on 1 to 5 DPI, evaluated for sgRNA, and an area under the
120 curve was calculated per animal to determine the cumulative amount of virus shed. We observed
121 a decrease in the total amount of respiratory shedding for both vaccinated groups compared to
122 control animals, although this was statistically significant for B.1.1.7 only (Figure 1i, p=0.0115,
123 Mann-Whitney test).
124



125

126 **Figure 2. Pulmonary effects of direct intranasal challenge with SARS-CoV-2 variants**
127 **B.1.1.7 and B.1.351 in Syrian hamsters at 5 DPI.** a-d. Gross pathology of hamster lungs; a/c.
128 Normal lungs. b/d. Multifocal and focally extensive areas of consolidation. e-h. H&E staining,

20x; e/g. No pathology. f/h. Focally extensive areas of bronchointerstitial pneumonia. i-l. H&E staining, 200x; i/k. No pathology. j/l. Bronchointerstitial pneumonia with alveolar histiocytosis, fibrin and edema. m-p. IHC staining against N protein SARS-CoV-2 (brown). m/o. No staining. n/p. Staining of bronchiolar epithelial cells, type I&II pneumocytes and rare macrophages.

We investigated the presence of the AA substitutions Q677H and R682W observed in 88-89% of the spike protein of our B.1.351 stock in swabs and lung tissue obtained from control animals challenged with B.1.351. Whereas we did find these substitutions in swabs obtained 1 DPI, they were not present in swabs obtained at 5 DPI. Likewise, the substitutions were only found in lung tissue of one out of four control hamsters (Table 2).

AA substitutions	Presence in swabs (1 DPI, N=5)	Presence in swabs (5 DPI, N=3)	Presence in lungs (N=4)
Q677H	44.1-65.7%	0%	0 (N=3), 81% (N=1)
R682W	44.9-65.8%	0%	0 (N=3), 81% (N=1)

Table 2. Presence of substitutions Q677H and R682W in swabs and lung tissue of hamsters directly inoculated with B.1.351.

This study demonstrates efficacy of the ChAdOx1 nCoV-19 vaccine against circulating variants of concern in the SARS-CoV-2 Syrian hamster model. The Syrian hamster SARS-CoV-2 infection model is characterized by natural susceptibility to SARS-CoV-2 and development of a robust upper and lower respiratory tract infection[6]. The hamster model has been successfully used for the preclinical development of several vaccines including the Ad26 and mRNA-1273 vaccines by Janssen[7] and Moderna[8], respectively. Several groups have reported the effect of spike protein substitutions observed in B.1.1.7 and B.1.351 VOCs on the virus neutralizing capacity of serum obtained from vaccinated or convalescent individuals. In general, these studies conclude that the substitutions found in the B.1.1.7 spike protein have limited to no effect on virus neutralization titres[9–15]. Data from a UK phase III trial taken from a time when B.1.1.7

153 predominated, showed minimal impact on ChAdOx1 nCoV-19 vaccine efficacy[15]. Likewise, in
154 an observational study of vaccine effectiveness in adults aged over 70 years in the UK, a single
155 dose of either ChAdOx1 nCoV-19 or the Pfizer/BioNTech vaccine BNT162b2 reduced
156 hospitalization in elderly adults with co-morbidities by 80%[16]. In contrast, the substitutions
157 found in the B.1.351 spike protein (Table 1) result in a significant reduction of virus neutralizing
158 capacity with pseudotype or infectious virus neutralization assays[9–15,17,18]. In a phase II study of
159 ChAdOx1 nCoV-19 in South Africa in 2000 adults with a median age of 31 years, vaccine
160 efficacy against mild to moderate disease was reduced when the virus recovered after infection
161 was B.1.351 (19 cases in the vaccinated group and 20 in the placebo group)[19]. Vaccine efficacy
162 against severe disease could not be determined as no severe cases occurred in this young cohort.
163 The South African arm of the ENSEMBLE study which tested vaccine efficacy after a single
164 dose of Janssen's COVID-19 vaccine candidate enrolled 6,576 participants in South Africa, out
165 of a total of 43,783 in multiple countries, with 34% of participants across the study aged over 60
166 years. Vaccine efficacy against moderate to severe disease was 64% (CI 41.2%, 78.7%) in South
167 Africa compared to 72% (CI 58.2%, 81.7%) in the USA at 28 days post vaccination[20]. Efficacy
168 of the vaccine against severe to critical disease was 81.7% in South Africa, which was similar to
169 the reported 85.9% and 87.6% in the USA and Brazil, respectively[20]. Vaccine efficacy against
170 mild disease was not reported. These clinical trial results are consistent with the findings of the
171 preclinical study reported here; ChAdOx1 nCov-19 may be less effective at reducing upper
172 respiratory tract infection caused by B.1.351 than by B.1.1.7, consistent with reduced efficacy
173 against mild disease. However, complete protection against lower respiratory tract disease was
174 observed in this challenge study, consistent with protection against severe disease. Based on our

175 data, we hypothesize that the currently available vaccines will likely still protect against severe
176 disease and hospitalization caused by VOC B.1.351.
177 Limited data on the immunological determinants of protection are available, however recent data
178 from rhesus macaques indicate that relatively low neutralizing antibody titers are sufficient for
179 protection against SARS-CoV-2, and that cellular immune responses may contribute to
180 protection if antibody responses are suboptimal[21]. Induction of binding and neutralizing
181 antibodies as well as SARS-CoV-2 cellular spike protein-specific T cell responses after
182 vaccination have been reported[22,23] and most SARS-CoV-2 specific T cell epitopes in both
183 convalescent and vaccinated individuals are not affected by the AA substitutions found in the
184 spike protein of the B.1.1.7 and B.1.351 variants[24]. Protection against severe COVID-19 disease
185 might be mediated by T cells and would therefore not be different between the current variants.
186 However, as T cell-mediated protection can only act after the initial infection has occurred, mild,
187 polymerase chain reaction (PCR)-positive disease may still occur in the upper respiratory tract.
188 It should be noted that the B.1.351 virus stock used to challenge hamsters contained two
189 additional non-fixed AA substitutions; Q677H and R682W at 88% and 89%, respectively.
190 Interestingly, the relative presence of these two AA substitutions was markedly reduced at 1 DPI
191 and absent on 5 DPI. In addition, they were only present in lung tissue of one control hamster at
192 5 DPI, suggesting that they are rapidly selected against in the SARS-CoV-2 hamster model over
193 the course of infection. Nonetheless, since the substitutions thought to be important in immune
194 evasion, such as E484K[18], are still present in the virus stock, efficient replication and lung
195 pathology was observed in infected hamsters, we do not believe the presence of these additional
196 AA substitutions present as a quasispecies affect the data interpretation.

197 Based on the current studies and healthcare priorities in real-world settings, we believe it is
198 essential to focus on prevention of moderate to severe disease requiring hospitalization. We show
199 that ChAdOx1 nCoV-19 vaccination resulted in complete protection against disease in hamsters.
200 As implied by the data presented by Janssen[20], viral vectored vaccines may provide substantial
201 protection against lower respiratory tract infection caused by the B.1.351 variant and subsequent
202 hospitalization and death. With the ongoing evolution of SARS-CoV-2, the readily available and
203 cost-effective hamster model allows rapid evaluation of the protective efficacy of novel VOCs.
204 In addition, it will allow rapid preclinical benchmarking of existing vaccines against preclinical
205 vaccines with updated antigen designs.
206

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Author contributions: N.v.D. and V.J.M. designed the studies, S.C.G. and T.L. designed and provided the vaccine, R.J.F., N.v.D., D.R.A., C.K.Y, J.R.P., M.G.H., J.E.S., B.N.W., T.T., K.B., S.L.A., S.R, B.J.S., D.L., C.M., G.S, E.d.W., and V.J.M. performed the experiments, R.J.F., N.v.D., D.R.A., C.K.Y, J.R.P., M.G.H., J.E.S., S.L.A., C.M., G.S, and V.J.M. analyzed results, R.J.F., N.v.D and D.R.A. wrote the manuscript, all co-authors reviewed the manuscript.;

Competing interests: S.C.G. is a board member of Vaccitech and named as an inventor on a patent covering the use of ChAdOx1-vector-based vaccines and a patent application covering a SARS-CoV-2 (nCoV-19) vaccine (UK patent application no. 2003670.3). T.L. is named as an inventor on a patent application covering a SARS-CoV-2 (nCoV-19) vaccine (UK patent application no. 2003670.3). The University of Oxford and Vaccitech, having joint rights in the vaccine, entered into a partnership with AstraZeneca in April 2020 for further development, large-scale manufacture and global supply of the vaccine. Equitable access to the vaccine is a key component of the partnership. Neither Oxford University nor Vaccitech will receive any royalties during the pandemic period or from any sales of the vaccine in developing countries. All other authors declare no competing interests.

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Materials and Methods

307 *Ethics Statement*

308 All animal experiments were conducted in an AAALAC International-accredited facility and
309 were approved by the Rocky Mountain Laboratories Institutional Care and Use Committee
310 following the guidelines put forth in the Guide for the Care and Use of Laboratory Animals 8th
311 edition, the Animal Welfare Act, United States Department of Agriculture and the United States
312 Public Health Service Policy on the Humane Care and Use of Laboratory Animals.
313 The Institutional Biosafety Committee (IBC) approved work with infectious SARS-CoV-2 virus
314 strains under BSL3 conditions. Virus inactivation of all samples was performed according to
315 IBC-approved standard operating procedures for the removal of specimens from high
316 containment areas.

317 *Cells and virus*

318 SARS-CoV-2 variant B.1.351 (hCoV-19/South African/KRISP-K005325/2020,
319 EPI_ISL_678615) was obtained from Dr. Tulio de Oliveira and Dr. Alex Sigal at the Nelson R
320 Mandela School of Medicine, UKZN. SARS-CoV-2 variant B.1.1.7 (hCoV-
321 19/England/204820464/2020, EPI_ISL_683466) was obtained from Public Health England via
322 BEI. Virus propagation was performed in VeroE6 cells in DMEM supplemented with 2% fetal
323 bovine serum, 1 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin (DMEM2).
324 VeroE6 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 1 mM L-
325 glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin. Mycoplasma testing is performed at
326 regular intervals and no mycoplasma was detected.

327 *Animal Experiments*

328 ChAdOx1 nCoV-19 was formulated as previously described[25]. Four groups of 10, 4-6-week-old
329 female Syrian hamsters (Envigo Indianapolis, IN) were vaccinated with 2.5 x 10^8 infectious units

330 of ChAdOx1 nCoV-19 vaccine or ChAdOx1-GFP delivered intramuscularly in two 50 µL doses
331 into the posterior thighs 30 days prior to challenge. Five days prior to challenge a blood sample
332 was collected via the retro-orbital plexus under isoflurane anesthesia and spun at 2000 g for 10
333 min to obtain serum. Two groups (10 ChAdOx1 nCoV-19 vaccinated and 10 ChAdOx1 GFP
334 vaccinated hamsters) were challenged with 10^4 $TCID_{50}$/mL B.1.1.7 diluted in sterile Dulbecco's
335 Modified Eagle's media (DMEM), in a 40 µL bolus delivered intranasally, one-half into each
336 nostril. Two other groups (10 ChAdOx1 nCoV-19 vaccinated and 10 ChAdOx1 GFP vaccinated
337 hamsters) were similarly challenged with B.1.351 also diluted in sterile DMEM. Weights were
338 recorded daily until 14 DPI. Oropharyngeal swabs were collected daily in 1 mL of DMEM2 up
339 until 5 DPI. On 5 DPI 4 animals from each group were euthanized. The lungs were excised,
340 weighed, and photographed, and samples taken for qRT-PCR analysis, virus titrations and
341 histopathology. The remaining six animals in each group were monitored daily for signs of
342 disease and weighed until 14 DPI.

343 *Virus titration*

344 Lung sections were weighed and homogenized in 1 mL of DMEM. Virus titrations were
345 performed by end-point titration of 10-fold dilutions of virus swab media or tissue homogenates
346 on VeroE6 cells in 96-well plates. When titrating tissue homogenate, the top 2 rows of cells were
347 washed 2 times with PBS prior to the addition of a final 100 µl of DMEM2. Cells were incubated
348 at 37°C and 5% CO2. Cytopathic effect was read 6 days later.

349 *Virus neutralization*

350 Sera were heat-inactivated (30 min, 56 °C). After an initial 1:10 dilution of the sera, two-fold
351 serial dilutions were prepared in DMEM2. 100 $TCID_{50}$ of SARS-CoV-2 variant B.1.1.7 or
352 B.1.351 was added to the diluted sera. After a 1hr incubation at 37°C and 5% CO_2, the virus-

353 serum mixture was added to VeroE6 cells. The cells were incubated for 6 days at 37°C and 5%
354 CO_2 at which time they were evaluated for CPE. The virus neutralization titer was expressed as
355 the reciprocal value of the highest dilution of the serum that still inhibited virus replication.
356 *RNA extraction and quantitative reverse-transcription polymerase chain reaction*
357 RNA was extracted from oropharyngeal swabs using the QiaAmp Viral RNA kit (Qiagen)
358 according to the manufacturer's instructions and following high containment laboratory
359 protocols. Lung samples were homogenized and extracted using the RNeasy kit (Qiagen)
360 according to the manufacturer's instructions and following high containment laboratory
361 protocols. A viral sgRNA[26] specific assay was used for the detection of viral RNA. Five µL of
362 extracted RNA was tested with the Quantstudio 3 system (Thermofisher) according to
363 instructions from the manufacturer. A standard curve was generated during each run using
364 SARS-CoV-2 standards containing a known number of genome copies.
365 *Viral RNA sequencing*
366 For sequencing from viral stocks, sequencing libraries were prepared using Stranded Total RNA
367 Prep Ligation with Ribo-Zero Plus kit per manufacturer's protocol (Illumina) and sequenced on
368 an Illumina MiSeq at 2 x 150 base pair reads. For sequencing from swab and lung tissue, total
369 RNA was depleted of ribosomal RNA using the Ribo-Zero Gold rRNA Removal kit (Illumina).
370 Sequencing libraries were constructed using the KAPA RNA HyperPrep kit following
371 manufacturer's protocol (Roche Sequencing Solutions). To enrich for SARS-CoV-2 sequence,
372 libraries were hybridized to myBaits Expert Virus biotinylated oligonucleotide baits following
373 the manufacturer's manual, version 4.01 (Arbor Biosciences, Ann Arbor, MI). Enriched libraries
374 were sequenced on the Illumina MiSeq instrument as paired-end 2 X 151 base pair reads. Raw
375 fastq reads were trimmed of Illumina adapter sequences using cutadapt version 1.12[27] and then

376 trimmed and filtered for quality using the FASTX-Toolkit (Hannon Lab, CSHL). Remaining
377 reads were mapped to the SARS-CoV-2 2019-nCoV/USA-WA1/2020 genome (MN985325.1) or
378 hCoV-19/England/204820464/2020 (EPI_ISL_683466) or hCoV-19/SouthAfrica/KRISP-
379 K005325/2020 (EPI_ISL_678615) using Bowtie2 version 2.2.9[28] with parameters --local --no-
380 mixed -X 1500. PCR duplicates were removed using picard MarkDuplicates (Broad Institute)
381 and variants were called using GATK HaplotypeCaller version 4.1.2.0[29] with parameter -ploidy
382 2. Variants were filtered for QUAL > 500 and DP > 20 using bcftools.

383 *Expression and purification of SARS-CoV-2 S and receptor binding domain*

384 Protein production was performed as described previously[30,31]. Expression plasmids encoding the
385 codon optimized SARS-CoV-2 full length S and RBD were obtained from Kizzmekia Corbett
386 and Barney Graham (Vaccine Research Center, Bethesda, USA)[32] and Florian Krammer (Icahn
387 School of Medicine at Mt. Sinai, New York, USA)[33]. Expression was performed in Freestyle
388 293-F cells (Thermofisher), maintained in Freestyle 293 Expression Medium (Gibco) at 37°C
389 and 8% CO2 shaking at 130 rpm. Cultures totaling 500 mL were transfected with PEI at a
390 density of one million cells per mL. Supernatant was harvested 7 days post transfection, clarified
391 by centrifugation and filtered through a 0.22 µM membrane. The protein was purified using Ni-
392 NTA immobilized metal-affinity chromatography (IMAC) using Ni Sepharose 6 Fast Flow Resin
393 (GE Lifesciences) or NiNTA Agarose (QIAGEN) and gravity flow. After elution the protein was
394 buffer exchanged into 10 mM Tris pH8, 150 mM NaCl buffer (S) or PBS (RBD) and stored at −
395 80°C.

396 *ELISA*

397 ELISA was performed as described previously[25]. Briefly, maxisorp plates (Nunc) were coated
398 overnight at 4°C with 50 ng/well S or RBD protein in PBS. Plates were blocked with 100 µl of

399 casein in PBS (Thermo Fisher) for 1hr at RT. Serum diluted 1:6,400 was further 2-fold serially
400 diluted in casein in PBS was incubated at RT for 1hr. Antibodies were detected using affinity-
401 purified polyclonal antibody peroxidase-labeled goat-anti-monkey IgG (Seracare, 074-11-021) in
402 casein followed by TMB 2-component peroxidase substrate (Seracare, 5120-0047). The reaction
403 was stopped using stop solution (Seracare, 5150-0021) and read at 450 nm. All wells were
404 washed 4x with PBST 0.1% tween in between steps. Threshold for positivity was set at 2x OD
405 value of negative control (serum obtained from unvaccinated hamsters prior to start of the
406 experiment).

407 *Data availability statement*

408 Data have been deposited in Figshare

409

410 **References Materials and Methods**

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