1 **Safety and immunogenicity of the Ad26.COV2.S COVID-19 vaccine candidate: interim results**

2 **of a phase 1/2a, double-blind, randomized, placebo-controlled trial**

3

4 Jerald Sadoff[1$], MD; Mathieu Le Gars[1$], PhD; Georgi Shukarev[1], MD; Dirk Heerwegh[2], PhD;

5 Carla Truyers[2], PhD; Anne Marit de Groot[1], PhD; Jeroen Stoop[1], PhD; Sarah Tete[1], PhD; Wim

6 Van Damme[3], MD; Isabel Leroux-Roels[4], MD; Pieter-Jan Berghmans[5], MD; Murray Kimmel[6],

7 MD; Pierre Van Damme[8], MD; Jan de Hoon[9], MD, PhD, MsC; Williams Smith[10], MD; Kathryn

8 E. Stephenson[11], MD; Dan H. Barouch[11], MD; Stephen C. De Rosa[12], MD; Kristen W. Cohen[12],

9 PhD, M. Juliana McElrath[12], MD, PhD; Emmanuel Cormier, PhD[1]; Gert Scheper[1], PhD; Jenny

10 Hendriks[1], PhD; Frank Struyf[2], MD; Macaya Douoguih[1], MD, MPH; Johan Van Hoof[1], MD;

11 Hanneke Schuitemaker[1#], PhD

12

13 1 Janssen Vaccines & Prevention, Leiden, The Netherlands

14 2 Janssen Research & Development, Beerse, Belgium

15 3 Janssen Clinical Pharmacology Unit, Merksem, Belgium

16 4 CEVAC - University of Gent, Gent, Belgium

17 5 SGS Life Sciences, Antwerp, Belgium
18
19 6 Optimal Research LLC, Melbourne, Florida, USA

20 7 OR Peoria, Illinois, USA
21
22 8 Centre for the Evaluation of Vaccination, University of Antwerp, Antwerp, Belgium

23 9 Center for Clinical Pharmacology, University Hospitals Leuven, Leuven, Belgium

24 10 VRG and NOCCR, Knoxville, Tennessee, USA

25 11 Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston,

26 MA, USA

27 12 Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle,

28 Washington, USA

2

29    $ both authors contributed equally to this study; # corresponding author (hschuite@its.jnj.com;

30   +31646270638)

31   Word count overall = 4,160

32   Abstract word count = 538

33

2

1   **ABSTRACT**
2   **BACKGROUND**
3   The ongoing coronavirus disease (COVID)-19 pandemic caused by severe acute respiratory
4   syndrome coronavirus 2 (SARS-CoV-2) might be controlled by an efficacious vaccine. Multiple
5   vaccines are in development, but no efficacious vaccine is currently available.

6
7   **METHODS**
8   We designed a multi-center phase 1/2a randomized, double-blinded, placebo-controlled clinical
9   study to assesses the safety, reactogenicity and immunogenicity of Ad26.COV2.S, a non-
10 replicating adenovirus 26 based vector expressing the stabilized pre-fusion spike (S) protein of
11 SARS-CoV-2. Ad26.COV2.S was administered at a dose level of $5x10^{10}$ or $1x10^{11}$ viral particles
12 (vp) per vaccination, either as a single dose or as a two-dose schedule spaced by 56 days in healthy
13 adults (18-55 years old; cohort 1a & 1b; n= 402 and healthy elderly $\geq$65 years old; cohort 3;
14 n=394). Vaccine elicited S specific antibody levels were measured by ELISA and neutralizing
15 titers were measured in a wild-type virus neutralization assay (wtVNA). CD4+ T-helper (Th)1 and
16 Th2, and CD8+ immune responses were assessed by intracellular cytokine staining (ICS).

17
18 **RESULTS**
19 We here report interim analyses after the first dose of blinded safety data from cohorts 1a, 1b and
20 3 and group unblinded immunogenicity data from cohort 1a and 3. In cohorts 1 and 3 solicited
21 local adverse events were observed in 58% and 27% of participants, respectively. Solicited
22 systemic adverse events were reported in 64% and 36% of participants, respectively. Fevers
23 occurred in both cohorts 1 and 3 in 19% (5% grade 3) and 4% (0% grade 3), respectively, were
24 mostly mild or moderate, and resolved within 1 to 2 days after vaccination. The most frequent
25 local adverse event (AE) was injection site pain and the most frequent solicited AEs were fatigue,
26 headache and myalgia. After only a single dose, seroconversion rate in wtVNA (50% inhibitory
27 concentration - IC50) at day 29 after immunization in cohort 1a already reached 92% with GMTs
28 of 214 (95% CI: 177; 259) and 92% with GMTs of 243 (95% CI: 200; 295) for the $5x10^{10}$ and
29 $1x10^{11}$vp dose levels, respectively. A similar immunogenicity profile was observed in the first 15
30 participants in cohort 3, where 100% seroconversion (6/6) (GMTs of 196 [95%CI: 69; 560]) and
31 83% seroconversion (5/6) (GMTs of 127 [95% CI: <58; 327]) were observed for the $5x10^{10}$ or
32 $1x10^{11}$ vp dose level, respectively. Seroconversion for S antibodies as measured by ELISA (ELISA
33 Units/mL) was observed in 99% of cohort 1a participants (GMTs of 528 [95% CI: 442; 630) and

34   695 (95% CI: 596; 810]), for the $5x10^{10}$ or $1x10^{11}$ vp dose level, respectively, and in 100% (6/6

35   for both dose levels) of cohort 3 with GMTs of 507 (95% CI: 181; 1418) and 248 (95% CI: 122;

36   506), respectively. On day 14 post immunization, Th1 cytokine producing S-specific CD4+ T cell

37   responses were measured in 80% and 83% of a subset of participants in cohort 1a and 3,

38   respectively, with no or very low Th2 responses, indicative of a Th1-skewed phenotype in both

39   cohorts. CD8+ T cell responses were also robust in both cohort 1a and 3, for both dose levels.

40   **CONCLUSIONS**

41   The safety profile and immunogenicity after only a single dose are supportive for further clinical

42   development of Ad26.COV2.S at a dose level of $5x10^{10}$ vp, as a potentially protective vaccine

43   against COVID-19.

44   Trial registration number: NCT04436276

45   **INTRODUCTION**

46   The first cases of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory virus

47   coronavirus 2 (SARS-CoV-2) were reported in Wuhan, China, in December 2019.[1, 2] Since then,

48   the virus has infected millions of people globally. Infection with SARS-CoV-2 can result in a range

49   of clinical manifestations, varying from asymptomatic infection to severe acute respiratory distress

50   and death. To halt this pandemic, and to stop the pressure on health care systems and the negative

51   effects on the global economy, efficacious COVID-19 vaccines are urgently needed.

52   Several vaccine candidates are currently in different stages of development.[3–6] We have developed

53   Ad26.COV2.S, that is based on Janssen's replication incompetent adenovirus serotype 26 (Ad26)

54   vector, and a stabilized SARS-CoV-2 Spike (S) protein. This candidate was selected based on its

55   immunogenicity and manufacturability profile.[7] The Ad26 vector is also used for our Ebola

56   vaccine, and RSV, HIV and Zika vaccine candidates and Ad26-based vaccines are generally well

57   tolerated and highly immunogenic.[10]

58   We recently reported that a single dose of Ad26.COV2.S elicited strong immune responses in

59   rhesus macaques, and upon challenge with SARS-CoV-2, none of the vaccinated animals had

60   detectable viral load (VL) in bronchoalveolar lavage (BAL) and only one out of six had transient

61   and low VL in the nose.[5] Protective efficacy strongly correlated with the presence of virus

62   neutralizing activity in serum of the animals. In addition, we demonstrated that Ad26.COV2.S

63   provided protective immunity in a Syrian golden hamster severe disease model.[11]

64   These preclinical data supported the start of a Phase 1/2a randomized, double-blinded, placebo-

65   controlled clinical study to assesses the safety, reactogenicity and immunogenicity of

66   Ad26.COV2.S in one- or two-dose (8-week interval) schedules with 5×10^{10} or 1×10^{11} viral

67   particles (vp) per vaccination in adults 18–55 or $\geq$65 years of age. Here we report interim blinded

68   safety and reactogenicity data as well as group unblinded immunogenicity data obtained during

69   the first 4 weeks after the first vaccination. Based on these results, we have initiated a Phase 3

70   study that will evaluate the efficacy of a single vaccination of 5×10^{10} vp of Ad26.COV2.S (Trial

71   Number: NCT04505722).

72

73

74

75

METHODS

STUDY DESIGN AND PARTICIPANTS

76
77
78 We are performing a multicenter, randomized, double-blind, placebo-controlled Phase 1/2a trial
79 to evaluate safety, reactogenicity and immunogenicity of Ad26.COV2.S at $5x10^{10}$ or $1x10^{11}$ vp,
80 administered intramuscularly (IM) as single-dose or two-dose schedules, 8 weeks apart, in healthy
81 adults 18–55 years of age (cohort 1a (target n=375; interim safety and immunogenicity results post
82 dose 1 reported here) and cohort 1b (target n=25; interim safety results post dose 1 reported here))
83 and $\geq$65 years of age (cohort 3 (target n=375; interim post dose 1 safety on full cohort reported
84 here, as well as interim immunogenicity of first 15 participants that were 65–75 years of age)).
85 Enrollment of Cohort 2 will start later and will allow us to collect additional safety and
86 immunogenicity data (see Supplementary Table 1 for details on cohort 2). Overview of the clinical
87 trial design is given in Table 1 and in the flow chart in Figure 1.
88 Ad26.COV2.S is a recombinant, replication-incompetent Ad26 vector[12] encoding the full length
89 and stabilized SARS-CoV-2 S protein[7] derived from the first clinical isolate of the Wuhan strain
90 (Wuhan, 2019, whole genome sequence NC_045512). The study is performed at multiple clinical
91 sites in Belgium and the United States. A list of inclusion and exclusion criteria is provided at
92 ClinicalTrials.gov (Trial registration number: NCT04436276). All participants were screened for
93 COVID-19 by the collection of nasal samples for PCR, which, if positive, would exclude them,
94 and by locally available serological assays for detection of previous infection with SARS-CoV-2
95 with a maximum of 25 seropositive participants allowed between Cohort 1a and Cohort 3. The
96 study was reviewed and approved by local ethics committees (Comité d'Ethique Hospitalo-
97 Facultaire Sain-Luc, Université Catholique de Louvain on July 16, 2020) and institutional review
98 boards (IRB) (approval by Advarra IRB on June 29 and July 10, 2020, for New Orleans Center for
99 Clinical Research and Optimal Research sites, respectively). All participants provided written
100 informed consent before enrollment.

101

RANDOMIZATION AND BLINDING

102
103 We randomly assigned 405 eligible participants 18–55 years of age and 405 participants $\geq$65 years
104 of age to receive one or two vaccinations with either a $5x10^{10}$ or $1x10^{11}$ vp dose level of vaccine,
105 or placebo (1:1:1:1:1 per age group): $5x10^{10}$vp/$5x10^{10}$vp; $5x10^{10}$vp/placebo; $1x10^{11}$vp /$1x10^{11}$vp;
106 $1x10^{11}$vp/placebo; or placebo/placebo for the first and second dose, respectively (Figure 1).

107   Randomization was done by an Interactive Web Response System (IWRS) and stratified by site

108   using randomly permuted blocks. Participants and investigators remain blinded at individual

109   participant level throughout the study. Vaccine and placebo were provided in masked identical

110   syringes. Sponsor and statisticians were group-unblinded for the interim analysis when all

111   participants completed the day 29 visit or discontinued earlier. Safety results are presented here in

112   a blinded manner in order to prevent accidental unblinding.

113

114   **ENDPOINTS**

115   Endpoints to support the primary objectives of safety and reactogenicity of each dose schedule

116   were adverse events (AEs) for 28 days after each vaccination, local and systemic reactogenicity

117   for 7 days after each vaccination, and serious adverse events (SAEs) throughout the study. AEs

118   were graded according to FDA Guidance document "Toxicity Grading Scale for Healthy Adult

119   and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". The secondary

120   endpoint was humoral immune response to the S protein of SARS-CoV-2 as demonstrated by

121   Spike-specific enzyme-linked immunosorbent assay (ELISA) and by neutralizing titers against

122   wild type SARS-CoV-2 in a wtVNA, and cellular immune responses as measured by intracellular

123   cytokine staining (ICS) after stimulation with S peptide pools.

124

125   **PROCEDURES**

126   Participants received intramuscular (IM) injections of $5x10^{10}$ vp, $1x10^{11}$ vp Ad26.COV2.S or

127   placebo (0.9% saline) in a 1 mL volume in the deltoid muscle at Day 1. Solicited AEs were

128   collected on diary cards for 7 days post vaccination, unsolicited AEs for 28 days after vaccination

129   and SAEs throughout the course of the study. Safety data are included up to the cut-off dates of 27

130   August 2020 (cohort 1) and 7 September 2020 (cohort 3). Baseline seropositivity was assessed by

131   local clinical assays, and blood samples for serum chemistry, hematology, and for immune

132   responses were and will be collected at several timepoints throughout the study; urine samples for

133   pregnancy testing were collected before vaccination.

134   At baseline and on Day 29, Spike (S)-specific binding antibodies were measured by ELISA.

135   Seropositivity was defined as a titer >50.3 EU/mL. SARS-CoV-2 serum neutralizing antibody

136   titers were measured in a microneutralization wtVNA using the Victoria/1/2020 SARS-CoV-2

137   strain at Public Health England (PHE). Seropositivity in the wtVNA was defined as an IC_{50} titer

138 >58. SARS-CoV-2 S-specific T-cell responses were measured at baseline and on Day 15 by ICS

139 using two pools of S peptide pools of 15mers overlapping by 11 amino acids. A Th1 response was

140 characterized by CD4+ T cells expressing IFN-γ and/or IL-2 and not IL-4, IL-5 and/or IL13; a Th2

141 response was characterized by CD4+ T cells expressing IL-4, IL-5 and/or IL-13 and CD40L by

142 CD4+ T cells. All assays were conducted in a blinded fashion and are described in detail in the

143 Appendix.

144

145 **STATISTICAL METHODS**

146 This study was designed to assess safety and immunogenicity. Safety data were analyzed

147 descriptively in the full analysis set and immunogenicity data were analyzed in the per protocol

148 immunogenicity population. SARS-CoV-2 Spike (S)-binding antibody titers expressed as ELISA

149 Unit per milliliters (EU/mL), and neutralizing antibody titers in the wtVNA, expressed as the

150 reciprocal serum dilution neutralizing 50% of the test virus dose (50% inhibitory concentration

151 [IC_{50}]), are displayed on a log_{10} scale and described using geometric mean titers (GMT) and 95%

152 confidence intervals (95% CIs). For both assays, seroconversion was defined as having an antibody

153 titer above the lower limit of quantification (LLOQ) post vaccination if the baseline titer was below

154 the LLOQ, or a 4-fold increase over baseline post vaccination if the baseline titer was above the

155 LLOQ. ICS responses were described as percentage of total CD4+ or CD8+ T cell population.

156 Sample positivity was determined with a one-sided Fisher's exact test comparing non-stimulated

157 versus S peptide stimulated wells. LLOQ was 0.022% and non-quantifiable values were imputed

158 to LLOQ/2. Th1/Th2 ratio was calculated if the Th1 and/or Th2 responses were positive and above

159 2xLLOQ. If the Th1 or Th2 response from a participant was not fulfilling these criteria, the

160 Th1/Th2 ratio was considered >1 if a Th2 response could not be measured and <1 if a Th1 response

161 could not be measured.

162

163

8

164 **RESULTS**

165 **STUDY PARTICIPANTS**

166 Screening of participants started July 13, 2020, first vaccination of cohort 1a participants (age 18–

167 55) was initiated on July 22, 2020 and completed on August 4, 2020, and in cohort 1b participants

168 initiated on July 29, 2020 and completed on August 7, 2020. 575 volunteers were screened, of

169 whom 380 were enrolled in cohort 1a (377 vaccinated) and 28 were screened and 25 enrolled in

170 cohort 1b (25 vaccinated; Figure 1A). Seven (1.7%) participants were seropositive for SARS-CoV-

171 2 at screening, seropositivity rate for Ad26 will be reported later. Vaccinations of cohort 3

172 participants (age >65) were initiated on August 3, 2020 and first vaccinations were completed on

173 August 24, 2020. 660 volunteers were screened, of whom 405 were enrolled and 403 were

174 vaccinated (at the time of database extract, safety and reactogenicity data was available for 394

175 vaccinated participants; Figure 1B). Immunogenicity results of only the first 15 participants of

176 cohort 3 were available at time of this publication.

177 Baseline characteristics were broadly comparable across groups (Table 2).

178

179 **VACCINE SAFETY AND REACTOGENICITY OF Ad26.COV2.S**

180 Blinded safety data from cohort 1a, 1b and 3 were collected and analyzed. Solicited AEs were

181 collected on diary cards for 7 days post vaccination, unsolicited AEs for 28 days after vaccination

182 and SAEs throughout the course of the study. The study is ongoing, and the second immunization

183 of the primary regimen will not be completed at the time of this submission. To ensure the

184 participants and the investigators evaluating the participants remain blinded in this study until the

185 last dose of the primary vaccination regimen is given, the safety data are presented without group

186 unblinding in this interim report.

187 In cohorts 1a and 1b the investigator's assessment of reactogenicity is available for 402

188 participants, of whom 288 (72%) participants have reported solicited AEs (Table 3). Solicited local

189 AEs were reported in 235 (58%) participants – mostly grade 1/grade 2. Three participants reported

190 grade 3 pain/tenderness. The most frequent AE was injection site pain. Solicited systemic AEs

191 were reported for 258 (64%) participants, mostly grade 1/grade 2, with grade 3 systemic AEs

192 reported for 46 (11%) participants. The most frequent AEs were fatigue, headache and myalgia.

193 Fever was reported in 76 (19%) participants, with grade 3 fever reported in 22 (5%) participants.

194 All fevers occurred within 2 days of immunization and resolved within 1 to 2 days. Of the

9

195   participants who experienced grade 1 or 2 fevers, 83% utilized antipyretics at the onset of

196   symptoms. 91% of the participants who experienced grade 3 fevers utilized antipyretics at the

197   onset of symptoms. Overall, 98 participants have reported unsolicited AEs with 12 reporting grade

198   3 AEs (Table 4).

199   In cohort 3, at the time of database extract, investigator's assessment of reactogenicity is available

200   for 394 participants, of whom 183 (46%) participants have reported solicited AEs (Table 3).

201   Solicited local AEs were reported in 108 (27%) participants, most of which were grade 1/Grade 2,

202   with one participant reporting grade 3 swelling and erythema. The most frequent AE was injection

203   site pain. Solicited systemic AEs were reported in 140 (36%) participants, most of which were

204   grade 1/grade 2, with three participants reporting grade 3 AEs. The most frequent AEs were

205   headache, fatigue and myalgia. Mild or moderate fevers of grade 1 or 2 were reported in 14 (4%)

206   of participants only one of which was a grade 2. There were no high or other fevers that restricted

207   daily living activities (grade 3) reported in cohort 3. Overall, 46 (12%) participants have reported

208   unsolicited AEs. Four (1%) participants have reported unsolicited grade 3 AEs.

209   No grade 4 AEs, solicited or unsolicited, were reported in any cohort.

210   No participant discontinued the study due to an AE. There were two SAEs: one hypotension judged

211   by the investigator to not be vaccine related because of a past history of recurrent hypotension, and

212   one participant with fever who was hospitalized overnight because of suspicion of COVID-19, wo

213   recovered within 12 hours, the fever was subsequently judged by the investigator to be vaccine

214   related. For details see supplementary material.

215   While reactogenicity was acceptable in all groups, there was a trend for higher reactogenicity with

216   the higher vaccine dose (data not shown) and with younger age. This will be reported in more

217   detail upon group unblinding of the data.

218

219

220   **IMMUNOGENICITY OF Ad26.COV2.S**

221   Immunogenicity data for this interim analysis were unblinded by dose level. Antibodies against a

222   stabilized SARS-CoV-2 full length Spike protein were measured by ELISA. In cohort 1a, 94%

223   and 98% of the participants that received the $5x10^{10}$ and $1x10^{11}$ vp dose, respectively, at baseline

224   had EU/mL GMTs to SARS-CoV-2 S protein below the LLOQ. By Day 29 after vaccination,

225   GMTs had increased to respectively 528 (95% CI: 442; 630) and 695 (95% CI: 596; 810), with

10

226   99% seroconversion in each dose group (Figure 2A). Of the cohort 1a participants who were

227   seropositive at baseline, 7 out of 8 and 2 out of 3 demonstrated the preset criterion of a 4-fold

228   increase in binding antibody titer to be considered a vaccine responder, for the $5x10^{10}$ and $1x10^{11}$

229   vp dose level groups, respectively. The initial 15 enrolled participants in cohort 3 were

230   seronegative at baseline and had seroconverted by Day 29 post vaccination with GMTs of 507

231   (95% CI: 181; 1418) and 248 (95% CI: 122; 506), for the $5x10^{10}$ and $1x10^{11}$ vp dose level group,

232   respectively. GMTs of 899 were observed in the human convalescent serum (HCS) panel used in

233   this study, with an overlap in the 95% CI of the GMT for both dose level in each cohort.

234   In a subset of participants (n=50) for each of the $5x10^{10}$ and $1x10^{11}$ vp dose level group, SARS-

235   CoV-2 neutralizing antibody titers were measured by wtVNA. In cohort 1a, GMTs <LLOQ at

236   baseline for both dose groups had increased to IC_{50} GMTs of 214 (95% CI: 177; 259) and 243

237   (95% CI: 200; 295) for the $5x10^{10}$ and $1x10^{11}$ vp group, respectively, by Day 29. Similar results

238   were observed in the first 15 participants of cohort 3, with a GMT <LLOQ at baseline that had

239   increased to GMTs of 196 (95% CI: 69; 560) and 127 (95% CI: <LLOQ; 327) for the $5x10^{10}$ and

240    $1x10^{11}$ vp group, respectively, by Day 29 (Figure 2B). For comparison, a GMT of 522 was

241   measured in the HCS panel used in this analysis, with an overlap in the 95% CI of the GMT

242   between HCS panel and both dose groups and cohorts. Several serum samples from trial

243   participants reached the upper limit of quantification (ULOQ = 640) for the vaccine sample

244   analysis run and are currently being reanalyzed at higher dilution, which will raise the overall

245   GMT for each dose level. At Day 29 post vaccination, 98% (97/99) of participants in cohort 1a

246   were positive for neutralizing antibodies against SARS-CoV-2, independent of vaccine dose level

247   that was given. The seroconversion rate at day 29 was 92% for both the $5x10^{10}$ and $1x10^{11}$ vp

248   group in cohort 1a and, 100% (6/6) and 83% (5/6) for the $5x10^{10}$ and $1x10^{11}$ vp group in cohort 3.

249   Importantly, in cohort 1a, a total of 82% and 94% of participants in the $5x10^{10}$ and $1x10^{11}$ vp

250   group, respectively, reached titers greater than 100, indicative of a robust response induced in the

251   vast majority of the participants after a single vaccination with Ad26.COV2.S. The wtVNA and

252   ELISA titers as measured in cohort 1a samples highly correlated, with a Spearman correlation of

253   0.86 (p<0.001, data not shown). Data from a pseudovirus expressing SARS-CoV-2 S protein

254   neutralization assay are pending and will be included in the full publication of our post dose 1

255   interim analysis.

256

11

257   SARS-CoV-2 S-specific CD4+ and CD8+ T cell responses were characterized in a subset of study

258   participants at baseline and 15 days post vaccination. Previous studies with SARS-CoV and

259   MERS-CoV vaccines in preclinical models have suggested an association between a Th2 skewed

260   CD4+ T cell response with vaccine-associated enhanced respiratory disease (VAERD).[13–15] To

261   explore this theoretical risk of VAERD here, we assessed CD4+ Th1 and Th2 responses induced

262   by Ad26.COV2.S. Following stimulation with peptides covering the whole S protein, median

263   CD4+ Th1 responses increased from undetectable at baseline to a median of 0.08% (95% CI: 0.05;

264   0.16) and 0.11% (95% CI: 0.07; 0.16) 15 days post vaccination for the $5x10^{10}$ and $1x10^{11}$ vp group,

265   respectively, in cohort 1a participants, and from non-detectable at baseline to a median of 0.36%

266   (95% CI: 0.15; 0.89) and 0.13% (95% CI: 0.04; 0.50), respectively, in the first participants of

267   cohort 3 (Figure 2C). In cohort 1a, 76% (95% CI: 65; 86) and 83% (95% CI: 73; 91) of participants

268   had detectable Th1 responses for recipients of the $5x10^{10}$ and $1x10^{11}$ vp dose levels, respectively,

269   and in the first 15 participants of cohort 3, 100% (95% CI: 54; 100) and 67% (95% CI: 22; 96),

270   respectively. Only one participant in the $5x10^{10}$ vp group in cohort 1a had a detectable Th2

271   response. However, the Th1/Th2 ratio for this participant was 28.9, indicative of a Th1-skewed

272   phenotype. In all other participants that had measurable Th1 and/or Th2 responses, the Th1/Th2

273   ratio ranged from 1.0 to 68.5. Overall, these data indicate that Ad26.COV2.S induced Th1-skewed

274   responses in both age groups, suggestive for a low risk of VAERD.

275

276   S-specific CD8+ T cell responses were identified by the expression of IFN-γ and/or IL-2 cytokines

277   upon S peptide stimulation (Figure 2D). 15 days post vaccination, the median magnitude of S-

278   specific CD8+ T cell responses was 0.07% (95% CI: 0.03; 0.19) and 0.09% (95% CI: 0.05; 0.19)

279   for the $5x10^{10}$ and $1x10^{11}$ vp group, respectively, in cohort 1a, and 0.05% (95% CI: 0.02; 0.24)

280   and 0.07% (95% CI:0.02; 0.14), respectively, in cohort 3. 51% (95% CI: 39; 63) and 64% (95%

281   CI: 52; 75) of participants in cohort 1a had a positive CD8+ T cell response to S peptide stimulation

282   for the $5x10^{10}$ and $1x10^{11}$ vp group, respectively and 33% (95% CI: 4%; 78%) of participants of

283   both dose level groups in cohort 3 had a detectable vaccine induced CD8+ T cell response.

284

285

12

286 **DISCUSSION**

287 The interim analysis of our Phase 1/2a study shows that our vaccine candidate Ad26.COV2.S has

288 an acceptable safety and reactogenicity profile and is immunogenic at both a $5x10^{10}$ or $1x10^{11}$ vp.

289 Although the safety data in this interim report remain blinded, the overall occurrence independent

290 of dose level of solicited systemic AEs of 64% with a 19% fever rate (5% grade 3) in adults aged

291 18 to 55 (Cohort 1a and 1b) stands in contrast to the solicited systemic AEs of 36% with a 4%

292 fever rate (0% grade 3), found in the participants ≥ 65 years of age. This finding suggests that the

293 vaccine candidate is less reactogenic in older adults. The safety profile is acceptable at any age

294 given the seriousness of the disease the vaccine can potentially protect against and the nature of

295 the pandemic, especially in the elderly which is the most vulnerable population to COVID-19. We

296 did observe a trend for higher reactogenicity with the higher vaccine dose. More details on the

297 safety and reactogenicity profile will be provided immediately upon group unblinding of the study,

298 after vaccine dosing in cohort 1a and 3 has been completed.

299

300 A single dose of Ad26.COV2.S elicited strong humoral responses in the vast majority of vaccine

301 recipients. S-binding antibody titers as measured by ELISA, increased from baseline to Day 29

302 post vaccination in 99% of the participants in cohort 1a and 100% of the first participants in cohort

303 3, independent of the vaccine dose level that was given. Similarly, high response rates were

304 observed in a wtVNA. 29 days post vaccination, 98% of the participants had detectable

305 neutralizing antibodies. 92% of cohort 1a participants and respectively 6 out of 6, and 5 out of 6

306 recipients of the $5x10^{10}$ vp and $1x10^{11}$ vp dose level in cohort 3, seroconverted for SARS-CoV-2

307 neutralizing antibodies. In cohort 1a, depending on the dose level, 82% to 94% of the participants

308 had a neutralizing antibody titer above 100.

309

310 The lack of standards and the use of different immune assays by different vaccine developers

311 makes it difficult to compare the performance of COVID-19 vaccine candidates that are currently

312 in development. In addition, the level of immune response required to confer protection is

313 unknown and even the least immunogenic vaccine may still elicit sufficient immunity to be

314 protective. All other COVID-19 vaccines currently in development require two doses while

315 neutralizing antibody responses in all participants reported here were obtained after a single dose

316 of Ad26.COV2.S. The potency of a single vaccination with our Ad26.COV2.S COVID-19 vaccine

13

317 candidate is supported by our recently reported study in non-human primates where a single dose

318 provided complete protection against SARS-CoV-2 replication in the lung and near complete

319 protection against viral replication in the nose.[5] In this prior preclinical study, all sham vaccinated

320 control animals had detectable virus in both lung and nose for 7–14 days. In vaccinated non-human

321 primates, protection against SARS-CoV-2 infection was correlated with neutralizing antibody

322 titers.

323

324 Our clinical data indicate that Ad26.COV2.S at both dose levels induces a strong neutralizing

325 antibody response in the vast majority of the healthy young adult and older participants. Although

326 only a limited number of participants of cohort 3 were included in the analysis so far, comparable

327 immunogenicity of Ad26.COV2.S in adults aged 18–55 and adults aged 65–75 as observed here

328 is encouraging for elderly individuals, who are at higher risk for developing severe COVID-19.

329 Compared to COVID-19 human convalescent sera, the levels of binding and neutralizing

330 antibodies induced by Ad26.COV2.S are in the same range for most participants. However, the

331 significance of this comparison is not yet established due to the variability in GMTs in different

332 human convalescent serum panels, likely related to the variability of the composition of these

333 panels. Demographics such as age, disease severity, and time of sampling since disease onset and

334 the number of samples in the panel could have an impact on the GMTs of antibody, making a

335 comparison to GMTs in samples from vaccinated participants arbitrary.[16]

336

337 Previous experience with certain animal models of SARS-CoV and MERS-CoV vaccines have

338 raised a theoretical concern of VAERD.[13–15] An association between this safety concern and poor

339 neutralizing potency of humoral immunity and Th2-skewed cellular immune responses has been

340 suggested. Here we show that all Ad26.COV2.S elicited CD4+ T cell responses were Th1 skewed

341 with no or very low Th2 responses. Indeed, in all responders, the Th1/Th2 ratio was above 1, in

342 line with previous experience with our Ad26-based vaccine platform (data on file).[10] This robust

343 CD4+ Th1 response was accompanied by strong CD8+ T cell responses following vaccination.

344 Robust CD4+ Th1 and CD8+ T cell responses, in addition to strong humoral responses elicited by

345 Ad26.COV2.S minimizes the theoretical risk of VAERD.

346 An efficacious single-dose COVID-19 vaccine would have advantages over a two-dose vaccine in

347 terms of implementation, especially during a pandemic. If a single dose of Ad26.COV2.S protects

14

348   against SARS.CoV.2 infection or COVID-19, the durability of protection will be important to

349   characterize. Data on the durability of the immune response elicited by a single dose of

350   Ad26.COV2.S as well as on the immune response after a second dose of Ad26.COV2.S will

351   become available from our ongoing Phase 1/2a studies (Table 1). In previous studies with the

352   Ad26-based Zika vaccine, durability of neutralizing antibodies after a single dose, defined as

353   seropositivity, was at least 12 months in 56% of vaccine recipients and a second dose further

354   increased this percentage.[10] Obviously, durability of immunity is not only reflected in the

355   maintenance of neutralizing antibody titers but also in the quality of vaccine priming of the

356   immune system resulting in immune memory. To this end, we will study the ability of vaccinated

357   participants in our ongoing Phase 2a study (COV2001) to mount an anamnestic response to a very

358   low dose of vaccine as a surrogate for exposure to SARS-CoV-2.

359

360   The interpretation of the interim analysis presented here for immunogenicity is limited by the

361   relatively small sample size in cohort 3, the short follow-up, and the demographic characteristics

362   of the study population, which may limit the generalizability of our findings. However, additional

363   data will become available soon.

364

365   In conclusion, our interim analysis indicates that a single dose of Ad26.COV2.S, either $5x10^{10}$ vp

366   or $1x10^{11}$ vp, is safe, well tolerated and highly immunogenic. Based on similar immunogenicity of

367   both dose levels, we have selected the lower dose for further clinical evaluation. In our first Phase

368   3 study that has been initiated in the US, and that will later include sites in Africa, and South and

369   Central America (Trial Number: NCT04505722), we will evaluate the efficacy of a single dose of

370    $5x10^{10}$ vp of the Ad26.COV2.S vaccine candidate. An additional Phase 3 study is planned to assess

371   the efficacy and durability of immunity of a two-dose schedule with $5x10^{10}$ vp of the

372   Ad26.COV2.S vaccine.

373

374

375

376

15

387

388   **AUTHOR CONTRIBUTIONS**

389   The clinical protocol was developed by JVP. Investigators at the clinical sites collected clinical

390   data. Immunogenicity testing for interim analysis was performed at Fred Hutch Cancer Research

391   Center, USA, Public Health England, UK and Nexelis, Canada. MLG, JS, JH, DH, CT, GS, DB,

392   FS, MD, JVH and HS were involved in data analysis and interpretation. All authors jointly wrote

393   the manuscript, made the decision to submit the manuscript for publication, attest to the integrity

394   of the study, the completeness and accuracy of the interim data, and the fidelity of the study to the

395   protocol.

396

397

398   **DISCLOSURES**

399   Janssen Pharmaceuticals and the US Army have a cooperative research agreement (Cooperative

400   Research and Development Agreement, CRADA). Under the terms of the agreement, Janssen may

401   provide financial resources to support work; there is no personal financial benefit from the

402   arrangement. JS, MLG, GS, DH, CT, MG, JS, ST, EC, GS, JH, FS, MD, JVH and HS are all

403   employees of Janssen Pharmaceuticals and may be Johnson & Johnson stockholders. .

16

REFERENCES

1. WHO . Disease outbreak news. 2020. Emergencies preparedness, response. Pneumonia of unknown origin – China.https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/ 5 January, Accessed 12 Jan 2020.

2. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 2020;382:727–733.

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; 396:467–478. DOI: 10.1016/S0140-6736(20)31604-4.

4. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA Vaccine against SARS-CoV-2 — Preliminary Report. New Engl J Med 2020; DOI: 10.1056/NEJMoa2022483. Online ahead of print. DOI: 10.1056/NEJMoa2022483.

5. Mercado NB, Zahn R, Wegmann F, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature 2020 DOI: 10.1038/s41586-020-2607-z. Online ahead of print. DOI: 10.1038/s41586-020-2607-z

6. Mulligan MJ, Lyke KE, Kitchin N, et al. Phase 1/2 study of COVID-19 RNA vaccine BNT162b1 in adults. Nature 2020; DOI: 10.1038/s41586-020-2639-4. Online ahead of print.

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. https://biorxiv.org/content/10.1101/2020.07.30.227470v1.

17

427   8. Anywaine Z, Whitworth H, Kaleebu P, et al. Safety and Immunogenicity of a 2-Dose

428   Heterologous Vaccination Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola

429   Vaccines: 12-Month Data From a Phase 1 Randomized Clinical Trial in Uganda and

430   Tanzania. J Infect Dis 2019; 220:4–56. DOI: 10.1093/infdis/jiz070

431   9. Williams K, Bastian AR, Feldman RA, et al. Phase 1 Safety and Immunogenicity Study

432   of a Respiratory Syncytial Virus Vaccine With an Adenovirus 26 Vector Encoding

433   Prefusion F (Ad26.RSV.preF) in Adults Aged ≥60 Years. J Infect Dis 2020; 222:979–

434   988. DOI: 10.1093/infdis/jiaa193

435   10. Barouch DH, Tomaka FL, Wegmann F, et al. Evaluation of a mosaic HIV-1 vaccine in a

436   multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial

437   (APPROACH) and in rhesus monkeys (NHP 13-19). Lancet 2018;392:232–243. DOI:

438   10.1016/S0140-6736(18)31364-3

439   11. Tostanoski LH, Wegmann F, Martinot AL, et al. Ad26 vaccine protects against SARS-

440   CoV-2 severe clinical disease in hamsters. Nat Med 2020;DOI: 10.1038/s41591-020-

441   1070-6. Online ahead of print.

442   12. Custers J., Kim D., Leyssen M., et al; Vaccines based on replication incompetent Ad26

443   viral vectors: Standardized template with key considerations for a risk/benefit

444   assessment. Vaccine, in press

445   13. Deming D, Sheahan T, Heise M, et al. Vaccine Efficacy in Senescent Mice Challenged

446   with Recombinant SARS-CoV Bearing Epidemic and Zoonotic Spike Variants. PLoS

447   Med 2018;DOI: 10.1371/journal.pmed.0030525

448   14. Iwata-Yoshkawa N, Uda A, Suzuki T, et al. Effects of Toll-Like Receptor Stimulation on

449   Eosinophilic Infiltration in Lungs of BALB/c Mice Immunized with UV-Inactivated

18

450   Severe Acute Respiratory Syndrome-Related Coronavirus Vaccine. J Virol

451   2014;88:8597–8614. DOI: 10.1128/JVI.00983-14

452   15. Yasui F, Kai C, Kitabatake M, et al. Prior Immunization with Severe Acute Respiratory

453   Syndrome (SARS)-Associated Coronavirus (SARS-CoV) Nucleocapsid Protein Causes

454   Severe Pneumonia in Mice Infected with SARS-CoV. J Immunol 2008;181:6337–6348.

455   DOI: 10.4049/jimmunol.181.9.6337

456   Moore JP, Klasse PJ. SARS-CoV-2 vaccines: 'Warp Speed' needs mind melds not warped

457   minds. J Virol 2020;94:e01083-20. doi: 10.1128/JVI.01083-20

458

459 **Tables and Figures**

460 **Table 1. COV1001 study design**

Cohort 1a (Adults ≥18 to ≤55 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57 (Vaccination 2)**
1	75	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp
2	75	Ad26.COV2.S 5×10^{10} vp	Placebo
3	75	Ad26.COV2.S 1×10^{11} vp	Ad26.COV2.S 1×10^{11} vp
4	75	Ad26.COV2.S 1×10^{11} vp	Placebo
5	75	Placebo	Placebo
Cohort 1b (Adults ≥18 to ≤55 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57 (Vaccination 2)**
1	5	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp
2	5	Ad26.COV2.S 5×10^{10} vp	Placebo
3	5	Ad26.COV2.S 1×10^{11} vp	Ad26.COV2.S 1×10^{11} vp
4	5	Ad26.COV2.S 1×10^{11} vp	Placebo
5	5	Placebo	Placebo
Cohort 2a (Adults ≥18 to ≤55 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57**
1-4	120	Ad26.COV2.S 5×10^{10} vp	No vaccination
5	15	Placebo	No vaccination
Cohort 2b (Adults ≥18 to ≤55 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57 (Vaccination 2)**
1-4	120	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp
5	15	Placebo	Placebo
Cohort 3 (Adults ≥65 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57 (Vaccination 2)**
1	75	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp
2	75	Ad26.COV2.S 5×10^{10} vp	Placebo
3	75	Ad26.COV2.S 1×10^{11} vp	Ad26.COV2.S 1×10^{11} vp
4	75	Ad26.COV2.S 1×10^{11} vp	Placebo
5	75	Placebo	Placebo
Total	**1,045**		

461

462

20

463 **Table 2. Baseline demographics**

Characteristics	C1a All Participants	C1b All Participants	C3 All Participants
N	377	25	394
Age (years)			
Median	34.00	42.00	69.00
Range	(18.0; 55.0)	(22.0; 52.0)	(65.0; 88.0)
Sex			
Female (%)	198 (52.5%)	13 (52.0%)	199 (50.5%)
Male (%)	179 (47.5%)	11 (44.0%)	195 (49.5%)
Undifferentiated	0	1 (4.0%)	0
Race			
White	342 (90.7%)	22 (88.0%)	385 (97.7%)
Black or African American	20 (5.3%)	0	3 (0.8%)
Asian	8 (2.1%)	2 (8.0%)	0
Native Hawaiian or other Pacific Islander	1 (0.3%)	0	0
American Indian or Alaska Native	3 (0.8%)	0	1 (0.3%)
Multiple	0	1 (4.0%)	0
Unknown	3 (0.8%)	0	1 (0.3%)
Not reported	0	0	4 (1.0%)
Ethnicity			
Hispanic or Latino	15 (4.0%)	2 (8.0%)	6 (1.5%)
Not Hispanic or Latino	358 (95.0%)	22 (88.0%)	385 (97.7%)
Unknown	1 (0.3%)	0	0
Not reported	3 (0.8%)	1 (4.0%)	3 (0.8%)
BMI (kg/m2)			
Mean (SD)	24.596 (3.1942)	24.456 (3.2223)	25.357 (2.8594)*
Range	(16.80; 29.90)	(18.80; 29.90)	(16.60; 29.90)*
SARS-CoV-2 Seropositivity status at baseline			
Positive	7 (1.9%)	0	3 (0.8%)

464
465 *N=393
466

467

21

468

469

470 **Table 3.** Summary of solicited AEs in all cohorts

Overall Summary of Solicited Adverse Events; Cohort 1a, 1b and 3 Full Analysis Set (COV1001 study)			
	All participants Cohort 1a	All participants Cohort 1b	All participants Cohort 3
Analysis full set	377	25	394
Post dose 1	377	25	394
Participants with one or more			
Solicited AE	269 (71.4%)	19 (76%)	183 (46.4%)
Solicited AE with worst Grade of 3 or higher	41 (10.9%)	5 (20%)	3 (0.8%)
Solicited local AE	222 (58.9%)	13 (52%)	108 (27.4%)
Solicited local AE with worst Grade of 3 or higher	3 (0.8%)	0 (0%)	1 (0.3%)
Solicited systemic AE	240 (63.7%)	18 (72%)	140 (35.5%)
Solicited systemic AE with worst Grade of 3 or higher	41 (10.9%)	5 (20%)	3 (0.8%)
Solicited systemic AEs considered to be related to study vaccine	238 (63.1%)	18 (72%)	136 (34.5%)
Solicited systemic AEs of Grade 3 or higher considered to be related to study vaccine	41 (10.9%)	5 (20%)	3 (0.8%)

471

472

473

474

475

476 **Table 4. Grade 3 unsolicited AEs in Cohort 1a and cohort 3**

Cohort	Age range	Sex	Preferred Term	Reported Term for the Adverse Event	Study Day of Start of Adverse Event	Study Day of End of Adverse Event	Serious Event	Causality	Action Taken with Study Treatment	Outcome of Adverse Event	Concomitant or Additional Tx Given
COHORT 1A	40-50	F	Blood pressure decreased	DECREASED BLOOD PRESSURE	1	4	Y	NOT RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	50-55	M	White blood cell count increased	WBC INCREASE	8	12	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 1A	18-25	F	Malaise	MALAISE	1	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 1A	18-25	F	Back pain	BACKPAIN	2	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	35-40	F	Hypotensive crisis	HYPOTENSIVE CRISIS	1	1	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	30-35	M	Insomnia	INSOMNIA	2	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 1A	18-25	M	Contusion	CONTUSION RIGHT ANKLE	5		N	NOT RELATED	DOSE NOT CHANGED	RECOVERING/RESOLVING	Y
COHORT 1A	30-35	M	Pyrexia	FEVER	1	2	Y	RELATED	DRUG WITHDRAWN	RECOVERED/RESOLVED	Y
COHORT 1A	25-30	F	Back pain	BACKPAIN	1	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	18-25	M	Dizziness	LIGHTHEADEDNESS	2	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 1A	18-25	F	Heat stroke	SUNSTROKE	13	13	N	NOT RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	40-45	F	Neck pain	NECK PAIN	1	16	N	NOT RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 3	65-70	F	Dizziness	DIZZINESS	6	7	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 3	65-70	F	Vomiting	VOMITING	6	7	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 3	75-80	M	Systolic hypertension	GRADE 3 SYSTOLIC HYPERTENSION	15		N	NOT RELATED	DOSE NOT CHANGED	RECOVERING/RESOLVING	N
COHORT 3	70-75	M	Hypertension	HYPERTENSION WORSENING	8		N	RELATED	DOSE NOT CHANGED	RECOVERING/RESOLVING	N
COHORT 3	65-70	F	Systolic hypertension	SYSTOLIC HYPERTENSION (GRADE 3)	1	1	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 3	65-70	F	Bradycardia	WORSENING OF BRADYCARDIA	1	1	N	NOT RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N

477

478

479

480

481

482

24

483 **Figure 1: Consort Flow charts for cohort 1a, cohort 1b and cohort 3**

484 **A**

Cohort 1a

575 volunteers were screened for eligibility

127 screen failures
29 other, not randomized
4 withdrawal by subject/study tests too hard
35 various other reasons*
*Only reasons occurring in more than 2 subjects are shown

380 volunteers were randomized

153 assigned to receive Ad26 5×10^{10}

150 assigned to receive Ad26 1×10^{11}

77 assigned to receive PL

152 received Ad26 5×10^{10}

148 received Ad26 1×10^{11}

77 received PL

2 Withdrawal by subject

2 Withdrawal by subject
1 Other

485

Cohort 1b

28 volunteers were screened for eligibility

3 not randomized not vaccinated (screen failure)

25 volunteers were randomized

10 assigned to receive Ad26 5×10^{10}

10 assigned to receive Ad26 1×10^{11}

5 assigned to receive PL

10 received Ad26 5×10^{10}

10 received Ad26 1×10^{11}

5 received PL

486

487 **B**

488

25

Cohort 3

```
660 volunteers were screened for eligibility
                    │
                    ├──────────────────────→  255 not randomized
                    │                          not vaccinated
                    ▼                          (screen failures)
        405 volunteers were randomized
                    │
      ┌─────────────┼─────────────┐
      ▼             ▼             ▼
161 assigned to   162 assigned to   82 assigned to receive
receive Ad26      receive Ad26      PL
5x10^10           1x10^11
      │             │             │
      ▼             ▼             ▼
156 received      158 received      80 received
Ad26 5x10^10      Ad26 1x10^11      PL
```

Legend to Figure 1 Participants were enrolled concurrently at Belgian and US sites. Participants were randomized in parallel in a 1:1:1:1:1 ratio to one of five vaccination groups to receive one or two IM injections of Ad26.COV2.S at two dose levels of either 5×10^{10} vp or 1×10^{11} vp, or placebo. For cohort 1 and 3, in the absence of clinically significant findings 24 hours after the first vaccination was administered to five sentinel participants (two per dose level and one placebo), another ten participants were vaccinated across all groups. Safety data up to Day 28 were then reviewed by an internal data review committee before the remaining participants were randomized.
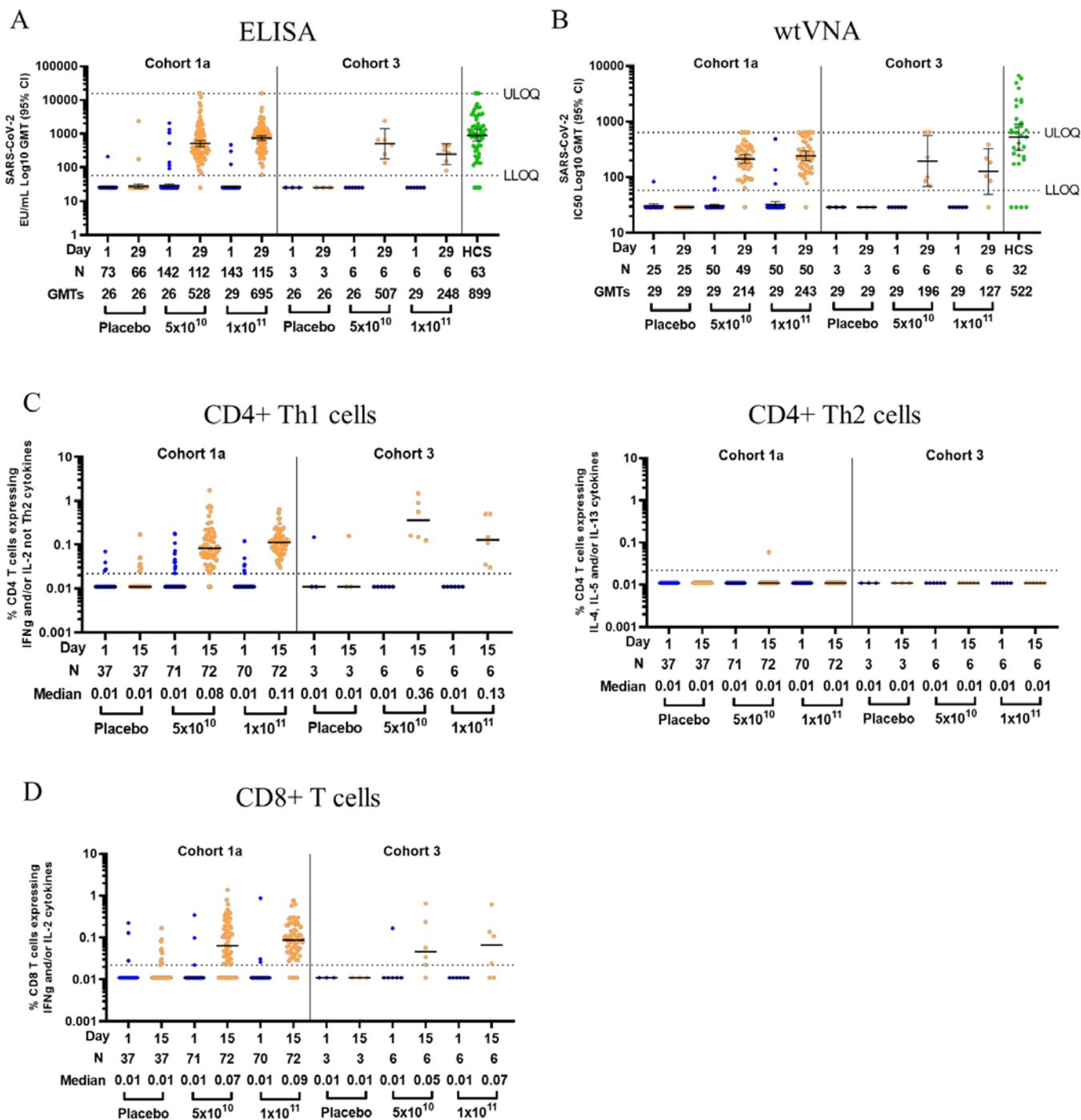
26

Figure 2: Immunogenicity of Ad26.COV2.S

Legend to Figure 2: (A) Log geometric mean titers (GMTs - as illustrated by the horizontal bars and the numbers below each timepoint) of SARS-CoV-2 binding antibodies in serum as measured by ELISA (ELISA Units per mL [EU/mL]), at baseline and at Day 29 post vaccination, among all

27

506   participants, according to schedule in cohort 1a and 3. Dotted lines indicate the lower limit of

507   quantification (LLOQ) and upper limit of quantification (ULOQ) of the assay, error bars indicate

508   95% confidence interval (CI). For values below the LLOQ, LLOQ/2 values were plotted.

509   (B) Log GMTs of serum SARS-CoV-2 neutralizing antibodies, measured by 50% neutralization

510   assay (IC_{50} Log GMT - as illustrated by the horizontal bars and the numbers below each timepoint),

511   at baseline and at Day 29 post vaccination, among a subset of participants, according to schedule,

512   in cohort 1a and 3. Dotted lines indicate the LLOQ and ULOQ of the assay run with the current

513   pre-dilution used for vaccine samples, error bars indicate 95% CI. For values below the LLOQ,

514   LLOQ/2 values were plotted.

515   (C) Expression of Th1 (IFN-γ and/or IL-2, and not IL-4, IL-5 and IL-13), and Th2 (IL-4 and/or

516   IL-5 and/or IL-13 and CD40L) cytokines by CD4+ T cells was measured by intracellular cytokine

517   staining (ICS). Median (as illustrated by the horizontal bars and the numbers below each timepoint)

518   and individual ICS responses to a SARS-CoV-2 S protein peptide pool in peripheral blood

519   mononuclear cells, at baseline and 15 days post vaccination, among a subset of participants from

520   cohort 1a and 3, according to schedule, are given. The Y-axis denotes the percentage of T cells

521   positive for the Th1 or Th2 cytokines. Dotted line indicates the LLOQ.

522   (D) Expression of IFN-γ and/or IL-2 cytokines by CD8+ T cells was measured by ICS. Median

523   (as illustrated by the horizontal bars and the numbers below each timepoint) and individual ICS

524   responses to SARS-CoV-2 S protein peptide pool in peripheral blood mononuclear cells, at

525   baseline and 15 days post vaccination, among a subset of participants from cohort 1a and 3,

526   according to schedule, are given. The Y-axis denotes the percentage of CD8+ T cells positive for

527   IFN-γ and/or IL-2 cytokines. Dotted line indicates the LLOQ.