

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**

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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 assess 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 5×10^{10} or 1×10^{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 ≥ 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 5×10^{10} and
29 1×10^{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 5×10^{10} or
32 1×10^{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 5×10^{10} or 1×10^{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.COVS.2.S at a dose level of 5×10^{10} vp, as a potentially protective vaccine
43 against COVID-19.

44 Trial registration number: NCT04436276

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INTRODUCTION

The first cases of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory virus coronavirus 2 (SARS-CoV-2) were reported in Wuhan, China, in December 2019.^{1, 2} Since then, the virus has infected millions of people globally. Infection with SARS-CoV-2 can result in a range of clinical manifestations, varying from asymptomatic infection to severe acute respiratory distress and death. To halt this pandemic, and to stop the pressure on health care systems and the negative effects on the global economy, efficacious COVID-19 vaccines are urgently needed.

Several vaccine candidates are currently in different stages of development.³⁻⁶ We have developed Ad26.COV2.S, that is based on Janssen's replication incompetent adenovirus serotype 26 (Ad26) vector, and a stabilized SARS-CoV-2 Spike (S) protein. This candidate was selected based on its immunogenicity and manufacturability profile.⁷ The Ad26 vector is also used for our Ebola vaccine, and RSV, HIV and Zika vaccine candidates and Ad26-based vaccines are generally well tolerated and highly immunogenic.¹⁰

We recently reported that a single dose of Ad26.COV2.S elicited strong immune responses in rhesus macaques, and upon challenge with SARS-CoV-2, none of the vaccinated animals had detectable viral load (VL) in bronchoalveolar lavage (BAL) and only one out of six had transient and low VL in the nose.⁵ Protective efficacy strongly correlated with the presence of virus neutralizing activity in serum of the animals. In addition, we demonstrated that Ad26.COV2.S provided protective immunity in a Syrian golden hamster severe disease model.¹¹

These preclinical data supported the start of a Phase 1/2a randomized, double-blinded, placebo-controlled clinical study to assesses the safety, reactogenicity and immunogenicity of Ad26.COV2.S in one- or two-dose (8-week interval) schedules with 5×10^{10} or 1×10^{11} viral particles (vp) per vaccination in adults 18–55 or ≥ 65 years of age. Here we report interim blinded safety and reactogenicity data as well as group unblinded immunogenicity data obtained during the first 4 weeks after the first vaccination. Based on these results, we have initiated a Phase 3 study that will evaluate the efficacy of a single vaccination of 5×10^{10} vp of Ad26.COV2.S (Trial Number: NCT04505722).

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METHODS

STUDY DESIGN AND PARTICIPANTS

We are performing a multicenter, randomized, double-blind, placebo-controlled Phase 1/2a trial to evaluate safety, reactogenicity and immunogenicity of Ad26.COV2.S at 5×10^{10} or 1×10^{11} vp, administered intramuscularly (IM) as single-dose or two-dose schedules, 8 weeks apart, in healthy adults 18–55 years of age (cohort 1a (target n=375; interim safety and immunogenicity results post dose 1 reported here) and cohort 1b (target n=25; interim safety results post dose 1 reported here)) and ≥ 65 years of age (cohort 3 (target n=375; interim post dose 1 safety on full cohort reported here, as well as interim immunogenicity of first 15 participants that were 65–75 years of age)). Enrollment of Cohort 2 will start later and will allow us to collect additional safety and immunogenicity data (see Supplementary Table 1 for details on cohort 2). Overview of the clinical trial design is given in Table 1 and in the flow chart in Figure 1.

Ad26.COV2.S is a recombinant, replication-incompetent Ad26 vector¹² encoding the full length and stabilized SARS-CoV-2 S protein⁷ derived from the first clinical isolate of the Wuhan strain (Wuhan, 2019, whole genome sequence NC_045512). The study is performed at multiple clinical sites in Belgium and the United States. A list of inclusion and exclusion criteria is provided at ClinicalTrials.gov (Trial registration number: NCT04436276). All participants were screened for COVID-19 by the collection of nasal samples for PCR, which, if positive, would exclude them, and by locally available serological assays for detection of previous infection with SARS-CoV-2 with a maximum of 25 seropositive participants allowed between Cohort 1a and Cohort 3. The study was reviewed and approved by local ethics committees (Comité d’Ethique Hospitalo-Facultaire Sain-Luc, Université Catholique de Louvain on July 16, 2020) and institutional review boards (IRB) (approval by Advarra IRB on June 29 and July 10, 2020, for New Orleans Center for Clinical Research and Optimal Research sites, respectively). All participants provided written informed consent before enrollment.

RANDOMIZATION AND BLINDING

We randomly assigned 405 eligible participants 18–55 years of age and 405 participants ≥ 65 years of age to receive one or two vaccinations with either a 5×10^{10} or 1×10^{11} vp dose level of vaccine, or placebo (1:1:1:1:1 per age group): 5×10^{10} vp/ 5×10^{10} vp; 5×10^{10} vp/placebo; 1×10^{11} vp / 1×10^{11} vp; 1×10^{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.

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125 **PROCEDURES**

126 Participants received intramuscular (IM) injections of 5×10^{10} vp, 1×10^{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.

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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 [IC₅₀]), are displayed on a log₁₀ 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.

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163

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

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, who
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.

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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 5×10^{10} and 1×10^{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

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 5×10^{10} and 1×10^{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 5×10^{10} and 1×10^{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 5×10^{10} and 1×10^{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 IC₅₀ GMTs of 214 (95% CI: 177; 259) and 243
237 (95% CI: 200; 295) for the 5×10^{10} and 1×10^{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 5×10^{10} and
240 1×10^{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 5×10^{10} and 1×10^{11} vp
248 group in cohort 1a and, 100% (6/6) and 83% (5/6) for the 5×10^{10} and 1×10^{11} vp group in cohort 3.
249 Importantly, in cohort 1a, a total of 82% and 94% of participants in the 5×10^{10} and 1×10^{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

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 5x10¹⁰ and 1x10¹¹ 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 5x10¹⁰ and 1x10¹¹ 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 5x10¹⁰ 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.

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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 5x10¹⁰ and 1x10¹¹ 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 5x10¹⁰ and 1x10¹¹ 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.

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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 5×10^{10} or 1×10^{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 5×10^{10} vp and 1×10^{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

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.COVS 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.COVS 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.COVS 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.COVS 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.COVS 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.COVS protects

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.COVS as well as on the immune response after a second dose of Ad26.COVS 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.COVS, either 5×10^{10} vp
366 or 1×10^{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 5×10^{10} vp of the Ad26.COVS vaccine candidate. An additional Phase 3 study is planned to assess
371 the efficacy and durability of immunity of a two-dose schedule with 5×10^{10} vp of the
372 Ad26.COVS vaccine.

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382

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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.

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397

398 **DISCLOSURES**

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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		

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463 **Table 2. Baseline demographics**

	C1a	C1b	C3
Characteristics	All Participants	All Participants	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/m ²)			
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%)

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465 *N=393

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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%)

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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

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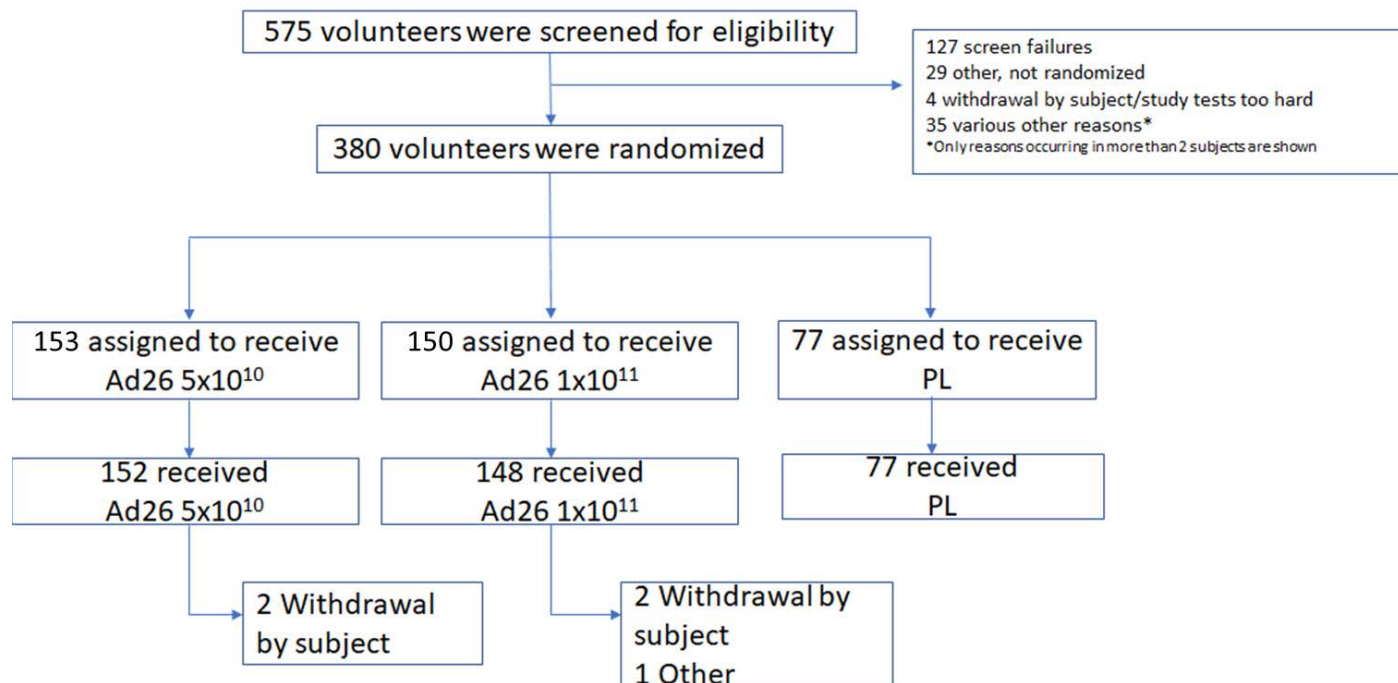
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483 **Figure 1: Consort Flow charts for cohort 1a, cohort 1b and cohort 3**

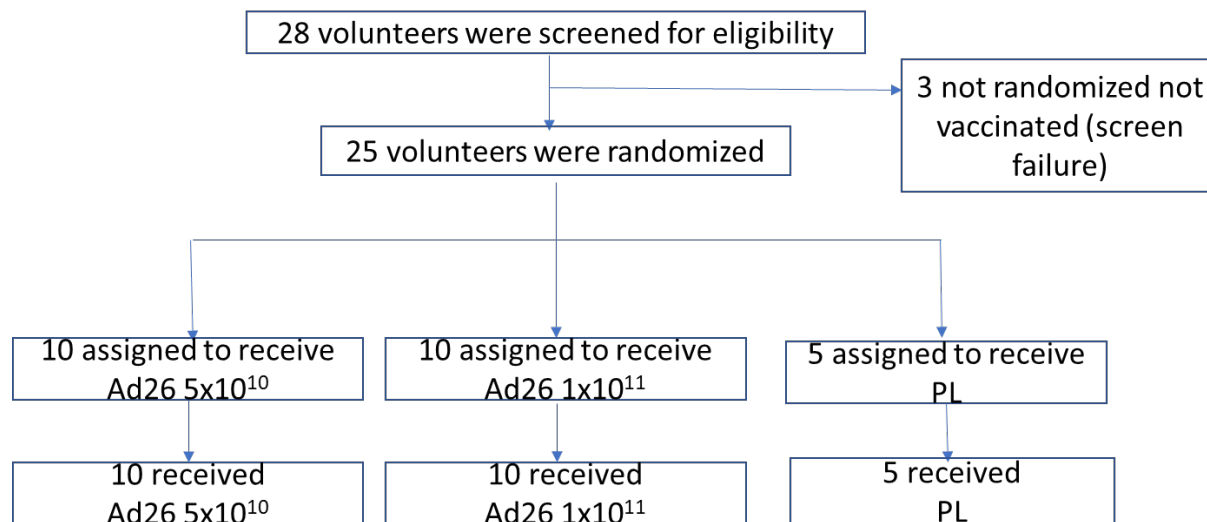
484 **A**

Cohort 1a



485

Cohort 1b

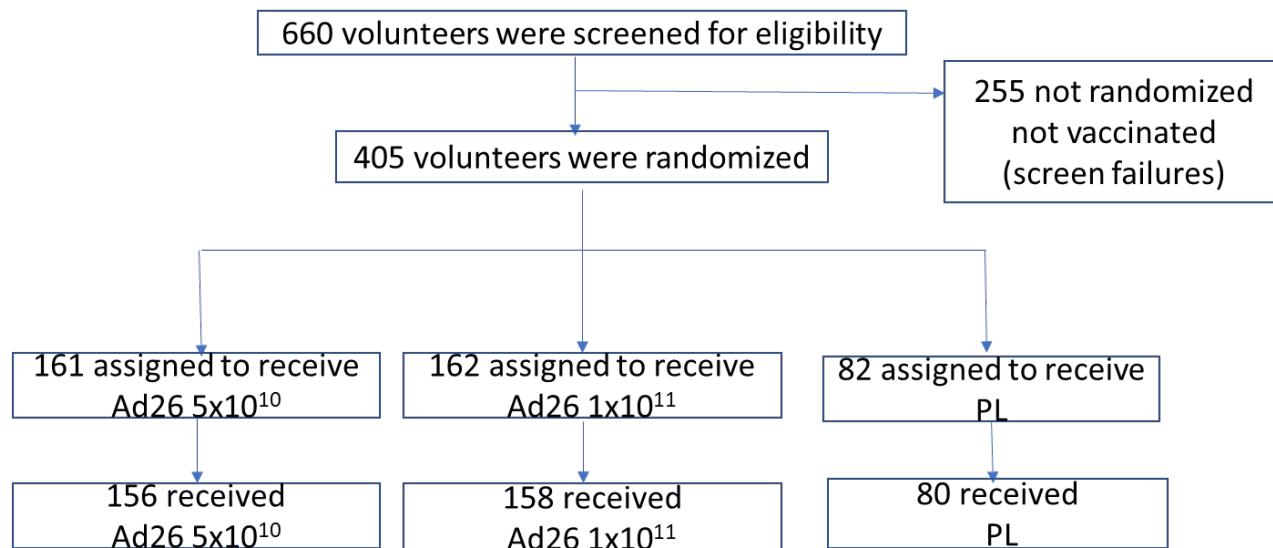


486

487 **B**

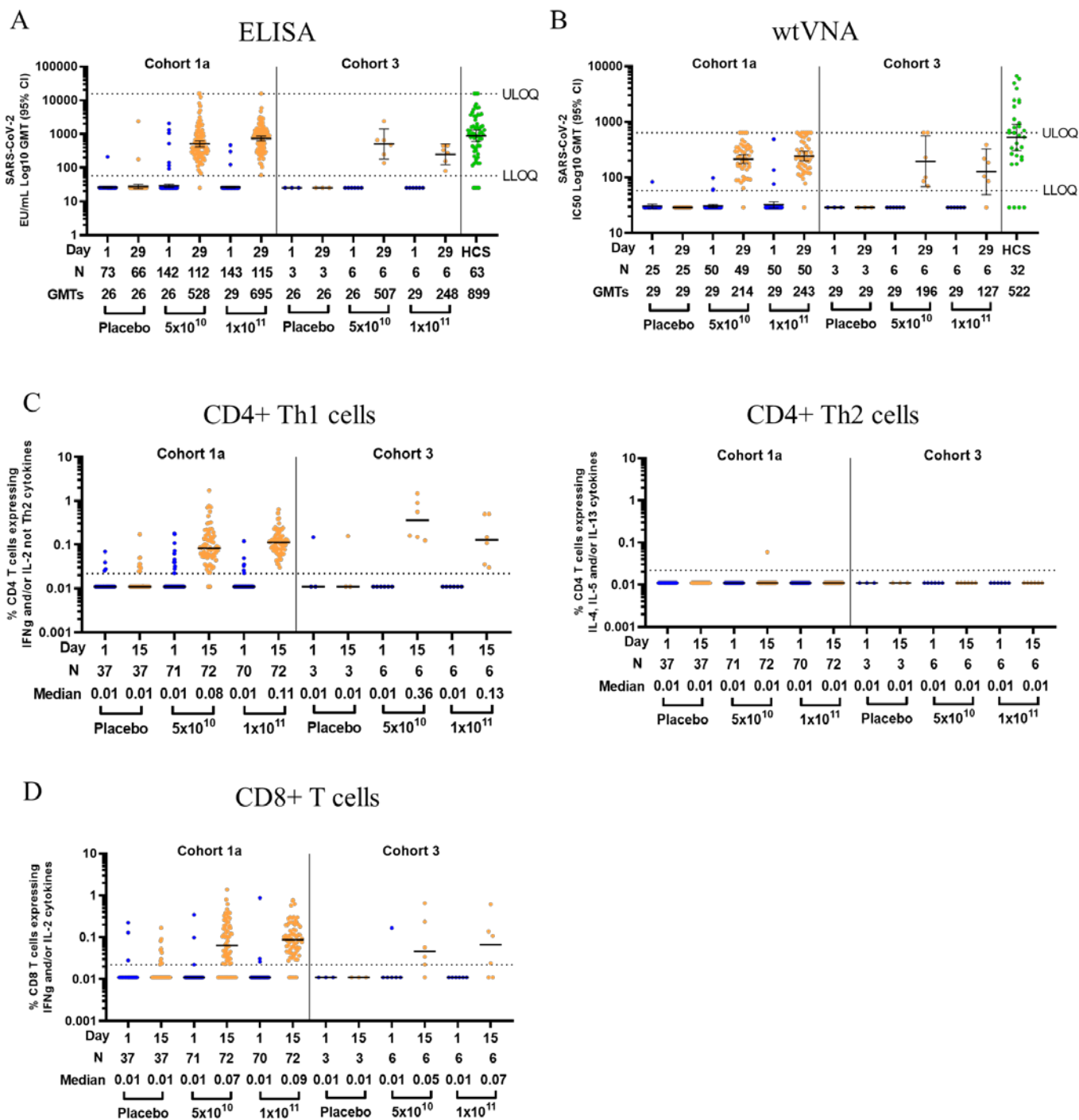
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Cohort 3



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490 **Legend to Figure 1** Participants were enrolled concurrently at Belgian and US sites. Participants
491 were randomized in parallel in a 1:1:1:1:1 ratio to one of five vaccination groups to receive one or
492 two IM injections of Ad26.COV2.S at two dose levels of either 5x10¹⁰ vp or 1x10¹¹ vp, or placebo.
493 For cohort 1 and 3, in the absence of clinically significant findings 24 hours after the first
494 vaccination was administered to five sentinel participants (two per dose level and one placebo),
495 another ten participants were vaccinated across all groups. Safety data up to Day 28 were then
496 reviewed by an internal data review committee before the remaining participants were randomized.
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500 **Figure 2: Immunogenicity of Ad26.COV2.S**



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 502
 503 **Legend to Figure 2:** (A) Log geometric mean titers (GMTs - as illustrated by the horizontal bars
 504 and the numbers below each timepoint) of SARS-CoV-2 binding antibodies in serum as measured
 505 by ELISA (ELISA Units per mL [EU/mL]), at baseline and at Day 29 post vaccination, among all

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 (IC₅₀ 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.