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Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial

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Summary

Background This is the first randomised controlled trial for assessment of the immunogenicity and safety of a candidate non-replicating adenovirus type-5 (Ad5)-vectored COVID-19 vaccine, aiming to determine an appropriate dose of the candidate vaccine for an efficacy study.

Methods This randomised, double-blind, placebo-controlled, phase 2 trial of the Ad5-vectored COVID-19 vaccine was done in a single centre in Wuhan, China. Healthy adults aged 18 years or older, who were HIV-negative and previous severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection-free, were eligible to participate and were randomly assigned to receive the vaccine at a dose of 1 × 10¹¹ viral particles per mL or 5 × 10¹⁰ viral particles per mL, or placebo. Investigators allocated participants at a ratio of 2:1:1 to receive a single injection intramuscularly in the arm. The randomisation list (block size 4) was generated by an independent statistician. Participants, investigators, and staff undertaking laboratory analyses were masked to group allocation. The primary endpoints for immunogenicity were the geometric mean titres (GMTs) of specific ELISA antibody responses to the receptor binding domain (RBD) and neutralising antibody responses at day 28. The primary endpoint for safety evaluation was the incidence of adverse reactions within 14 days. All recruited participants who received at least one dose were included in the primary and safety analyses. This study is registered with ClinicalTrials.gov, NCT04341389.

Findings 603 volunteers were recruited and screened for eligibility between April 11 and 16, 2020. 508 eligible participants (50% male; mean age 39.7 years, SD 12.5) consented to participate in the trial and were randomly assigned to receive the vaccine (1 × 10¹¹ viral particles n=253; 5 × 10¹⁰ viral particles n=129) or placebo (n=126). In the 1 × 10¹¹ and 5 × 10¹⁰ viral particles dose groups, the RBD-specific ELISA antibodies peaked at 656.5 (95% CI 575.2–749.2) and 571.0 (467.6–697.3), with seroconversion rates at 96% (95% CI 93–98) and 97% (92–99), respectively, at day 28. Both doses of the vaccine induced significant neutralising antibody responses to live SARS-CoV-2, with GMTs of 19.5 (95% CI 16.8–22.7) and 18.3 (14.4–23.3) in participants receiving 1 × 10¹¹ and 5 × 10¹⁰ viral particles, respectively. Specific interferon γ enzyme-linked immunospot assay responses post-vaccination were observed in 227 (90%, 95% CI 85–93) of 253 and 113 (88%, 81–92) of 129 participants in the 1 × 10¹¹ and 5 × 10¹⁰ viral particles dose groups, respectively. Solicited adverse reactions were reported by 183 (72%) of 253 and 96 (74%) of 129 participants in the 1 × 10¹¹ and 5 × 10¹⁰ viral particles dose groups, respectively. Severe adverse reactions were reported by 24 (9%) participants in the 1 × 10¹¹ viral particles dose group and one (1%) participant in the 5 × 10¹⁰ viral particles dose group. No serious adverse reactions were documented.

Interpretation The Ad5-vectored COVID-19 vaccine at 5 × 10¹⁰ viral particles is safe, and induced significant immune responses in the majority of recipients after a single immunisation.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused more than 12·1 million cases of COVID-19 worldwide, resulting in 551000 deaths and severe economic disruption. After the initial outbreak, with more than 80000 cases and 3000 deaths in China, COVID-19 has now spread to 216 countries and territories. Large numbers of cases and deaths are reported daily from Europe, the USA, Brazil, Russia, India, and many other countries. The current pandemic has highlighted the need for effective preventive solutions to reduce burden and spread of the disease.
Evidence before this study
We searched PubMed on July 16, 2020, for clinical trial reports with the terms “COVID-19” or “SARS-CoV-2”, “vaccine”, and “clinical trial”. Using the same terms, we also searched ClinicalTrials.gov for unpublished trials of COVID-19 vaccines. Except for the results of our earlier phase 1 study with the adenovirus type-5 (Ad5)-vectored vaccine and a phase 1 clinical trial with an mRNA vaccine (mRNA-1273) done in a small number of participants, no other COVID-19 vaccine data from clinical trials have been reported. We found registered trials with 11 candidate COVID-19 vaccines at ClinicalTrials.gov, including three recombinant protein-based vaccines, two viral vector-based vaccines, one DNA vaccine, two mRNA vaccines, two inactivated virus vaccines, and one autologous dendritic cell-based vaccine loaded with antigens from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The majority of the trials registered were in early phases; only ChAdOx1 nCoV-19 developed by the University of Oxford (Oxford, UK) is going to be evaluated in a phase 3 trial.

In the previously reported open-label, non-randomised, phase 1 trial, we found that the Ad5-vectored COVID-19 vaccine was tolerable and immunogenic in healthy adults. One dose of the vaccine induced rapid specific T-cell and humoral responses by 14 days.

Added value of this study
This study provides more evidence for the immunogenicity and safety of the Ad5-vectored COVID-19 vaccine in a larger population. To assess the vaccine in a more diverse population, we removed the age cap for the recruitment of participants for this phase 2 trial. Older individuals (ie, aged ≥55 years), many of whom often have chronic illness, have a high risk of serious illness and death associated with SARS-CoV-2 infection; thus, they are an important target population for a COVID-19 vaccine. Our results suggest a single-dose immunisation schedule of Ad5-vectored COVID-19 vaccine at 5×10¹⁰ viral particles is an appropriate regimen for healthy adults. Compared with the younger population, we found older people to have a significantly lower immune response, but higher tolerability, to the Ad5-vectored COVID-19 vaccine. Therefore, an additional dose might be needed to induce a better immune response in the older population, and this will be evaluated in a phase 2b trial.

Implications of all the available evidence
Evidence from this phase 2 study indicates the candidate Ad5-vectored COVID-19 vaccine has a good safety profile, with only mild, transient adverse events related to vaccination and no serious adverse events. Single-dose immunisation with the vaccine induced rapid onset of immune responses within 14 days and significant humoral and cellular immune responses within 28 days in the majority of the recipients. We are planning an international multicentre, randomised, double-blind, controlled phase 3 effectiveness trial to further evaluate the efficacy of the vaccine. We are in the midst of a global COVID-19 pandemic; thus, timely sharing of the results of clinical trials with candidate vaccines is critical.

Methods
Study design and participants
This randomised, double-blind, placebo-controlled, phase 2 trial of the Ad5-vectored COVID-19 vaccine was done in a single centre in Wuhan (Hubei province, China). The study was done in accordance with the Declaration of Helsinki and Good Clinical Practice. An independent data safety monitoring board was established before the start of the trial to provide oversight of the safety data during the study. The trial protocol was reviewed and approved by the National Medical Products Administration, China, and the institutional review board of the Jiangsu Provincial Center of Disease Control and Prevention. The protocol is available online.

Eligible participants were healthy adults aged 18 years or older, who were HIV-negative and previous SARS-CoV-2 infection-free, confirmed by commercial
Randomisation and masking
The Ad5-vectorised COVID-19 vaccine was developed by the Beijing Institute of Biotechnology (Beijing, China) and CanSino Biologics, and contained replication-defective Ad5 vectors expressing the full-length spike gene based on Wuhan-Hu-1 (GenBank accession number YP_009724390). The placebo contained the vaccine excipients only, with no viral particles. The experimental vaccines and placebos had identical packaging with a randomisation number on each vial as the only identifier. The vaccines of $5 \times 10^{11}$ and $5 \times 10^{10}$ viral particles and placebo were randomised at a 2:1:1 ratio. Eligible participants were sequentially assigned a randomisation number on each vial as the only identifier. The vaccines and placebos had identical packaging with a randomisation number on each vial as the only identifier. The vaccines of $5 \times 10^{11}$ and $5 \times 10^{10}$ viral particles and placebo were randomised at a 2:1:1 ratio. Eligible participants were sequentially assigned a randomisation number, according to a blocked randomisation list (block size 4) generated by an independent statistician using SAS software (version 9.4), and injected with an experimental vaccine or placebo labelled with the same number. Individuals involved in randomisation and masking had no involvement in the rest of the trial. Participants, investigators, and staff undertaking laboratory analyses were masked to group allocation.

Procedures
A single injection of the vaccines of $1 \times 10^{11}$ or $5 \times 10^{10}$ viral particles per mL, or placebo, were given to participants intramuscularly in the arm. Participants were monitored for 30 min post injection for immediate adverse reactions, and followed up for any injection site or systemic adverse reactions within 14 days and adverse events within 28 days post vaccination. Serious adverse events self-reported by participants were documented throughout the study. The detailed methods of the assays have been reported previously. In brief, blood samples were taken from participants at day 0 immediately before vaccination, and at days 14 and 28 post vaccination for the measurement of specific antibody responses against the receptor binding domain (RBD) using ELISA kits (Beijing Wantai BioPharm, Beijing, China). The detection limit for the RBD-specific ELISA antibody test was 1:40. The neutralising antibody responses live SARS-CoV-2 virus (strain SARS-CoV-2/human/CHN/Wuhan_IME-BJ01/2020, GenBank number MT291831.1) or a pseudovirus (a vesicular stomatitis virus pseudovirus system expressing the spike glycoprotein), and cellular immune responses before the vaccination and 28 days after the vaccination were also measured. The detection limits for the neutralising antibody tests to live SARS-CoV-2 virus and a pseudovirus were 1:8 and 1:10, respectively. Undetectable antibody titres in serum were assigned values of half the detection limits for calculation. The cellular immune responses of the expression of interferon (IFN) y stimulated by the overlapping peptide pool of spike glycoprotein were detected by enzyme-linked immunospot (ELISpot) assay (Mabtech, Stockholm, Sweden). Positive IFN-y-ELISpot response was defined as at least five spot-forming cells per $1 \times 10^5$ peripheral blood mononuclear cells, and a minimum of a two-times increase from baseline. Neutralising antibody titres against the vaccine vector Ad5 were measured with the serum neutralisation assay. The follow-ups were scheduled at days 14 and 28, and month 6 post vaccination for safety and immunogenicity assessment.

Outcomes
The primary objectives were to evaluate immunogenicity and safety of the Ad5-vectorised COVID-19 vaccine, and to determine a vaccine dose for a phase 3 efficacy study. The primary endpoint for safety evaluation was the incidence of adverse reactions within 14 days after the injection. The primary endpoints for immunogenicity were the geometric mean titres (GMTs) of RBD-specific ELISA antibody responses and neutralising antibody responses against live virus or pseudovirus at day 28 post vaccination. The secondary endpoints for immunogenicity were RBD-specific ELISA antibody responses at day 14 and month 6 (6 month data not yet available), and specific T-cell responses at day 28 post vaccination. Seroconversion of the humoral immune responses was also a secondary endpoint, and was defined as an increase in post-vaccination titre of at least four-times from baseline. The secondary safety outcomes included the occurrence of adverse events from days 0 to 28 after vaccination, and serious adverse events reported up to 6 months. Investigators did severity grading of the adverse events according to the standard guidelines issued by the China State Food and Drug Administration, and the causality with immunisation before unmasking. Stratified analysis of safety and immunogenicity of the participants was done based on the baseline Ad5 neutralising antibody titres with a cutoff at 1:200. Post-hoc analysis of the immune responses by age and sex, and the proportion of vaccine recipients with the composite endpoint of either positive cellular or seroconversion of humoral immune responses at day 28 post vaccination were presented.
imunogenicity data from the phase 1 study were available, using PASS software (version 11.0). For the RBD-specific antibody, 250 individuals in the $1 \times 10^{11}$ viral particles dose group and 125 individuals in the $5 \times 10^{10}$ viral particles dose group were able to provide at least a power of 90% to show a difference of the log-transferred titre of 0·176 with an SD of 0·4 between the dose groups, at a level of significance of 0·017, considering multiple comparisons.

Statistical tests were two-sided with an α value of 0·05, and analysed by an independent statistician using SAS (version 9.4). The primary immunogenicity analysis was done in the full-analysis cohort, including all participants who were injected and donated blood samples for antibody tests post vaccination, while the safety analysis was done in all enrolled participants who received the vaccination. Correlation analysis of the RBD-specific ELISA antibody and neutralising antibody was done, and the Pearson correlation coefficient was calculated. Antibody responses are reported as the GMT with 95% CI. ANOVA was used for log-transformed antibody titres, and the Wilcoxon rank-sum test for data that were not normally distributed. The χ² test or Fisher’s exact test was used for categorical data. Multiple comparisons were done if a significant difference across the treatment groups was noted, using Student Newman-Keuls test or Bonferroni-adjusted a value when relevant. This trial is registered with ClinicalTrials.gov, NCT04341389.

Role of the funding source
The funders of the study were involved in protocol design, but not in data collection, statistical analysis, data interpretation, or writing of the report. All the authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
603 volunteers were recruited and screened for eligibility between April 11 and 16, 2020 (figure 1). 95 individuals were excluded, leaving 508 eligible participants who consented to participate in the trial and were randomly assigned to vaccine or placebo. 253 were randomly assigned to the $1 \times 10^{11}$ viral particles dose group, 129 to the $5 \times 10^{10}$ viral particles dose group, and 126 to the placebo group. The mean age of the participants was 39·7 years (SD 12·5; range 18–83), with 309 (61%) individuals aged 18–44 years, 134 (26%) aged 45–54 years, and 65 (13%) aged 55 years or older across the treatment groups (table 1). The mean age of the participants was 39·7 years (SD 12·5; range 18–83), with 309 (61%) individuals aged 18–44 years, 134 (26%) aged 45–54 years, and 65 (13%) aged 55 years or older across the treatment groups (table 1). The mean age of the participants was 39·7 years (SD 12·5; range 18–83), with 309 (61%) individuals aged 18–44 years, 134 (26%) aged 45–54 years, and 65 (13%) aged 55 years or older across the treatment groups (table 1).

Table 1: Baseline characteristics

| Age, years | Vaccine at $1 \times 10^{11}$ vp (n=253) | Vaccine at $5 \times 10^{10}$ vp (n=129) | Placebo (n=126) |
|------------|-----------------------------------------|-----------------------------------------|-----------------|
| 18–44      | 152 (60%)                               | 80 (62%)                                | 77 (61%)        |
| 45–54      | 67 (26%)                                | 32 (25%)                                | 35 (28%)        |
| ≥55        | 34 (13%)                                | 17 (13%)                                | 14 (11%)        |
| Mean       | 40·0 (12·8)                             | 39·7 (12·1)                             | 39·2 (12·5)     |
| Sex        |                                         |                                         |                 |
| Male       | 126 (50%)                               | 64 (50%)                                | 64 (51%)        |
| Female     | 127 (50%)                               | 65 (50%)                                | 62 (49%)        |
| Body-mass index, kg/m² | 24·2 (2·8) | 24·2 (2·7) | 23·3 (2·6) |
| Underlying diseases |                     |                                         |                 |
| Yes        | 8 (3%)                                  | 8 (6%)                                  | 6 (5%)          |
| No         | 245 (97%)                               | 121 (94%)                               | 120 (95%)       |
| Pre-existing adenovirus type-5 neutralising antibody |             |                                         |                 |
| ≤1:200, titre | 127 (50%) | 54 (42%) | 61 (48%) |
| >1:200, titre | 126 (50%) | 75 (58%) | 65 (52%) |

Data are number of participants (%) or mean (SD). vp=viral particles.

Statistical analysis
This phase 2 trial was launched before the immunogenicity data from the phase 1 trial were obtained; therefore, the sample size was not calculated at the design stage. An overall sample size of 500 participants (n=250 in the $1 \times 10^{11}$ viral particles dose group; n=125 in the $5 \times 10^{10}$ viral particles dose group; and n=125 in the placebo group) was determined, based on expert opinion and the minimum sample size requirement in the technical guidelines for vaccine clinical trials issued by the National Medical Products Administration, China. We did an ex-post power calculation of this study after the

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Figure 1: Trial profile
vp=viral particles. *26 did not meet the inclusion criteria of negative IgM/IgG to severe acute respiratory syndrome coronavirus 2.
The baseline antibody titres of the participants are given in the appendix (p 1). RBD-specific ELISA antibody responses induced by the Ad5-vectored COVID-19 vaccine were detected from day 14 onwards, with GMTs of 94·5 (95% CI 80·5–110·8) and 85·1 (66·0–109·7) in the $1 \times 10^{11}$ and $5 \times 10^{10}$ viral particles dose groups, respectively (figure 2). At day 28, the RBD-specific ELISA antibodies peaked at 656·5 (575·2–749·2) in the $1 \times 10^{11}$ viral particles dose group and 571·0 (467·6–697·3) in the $5 \times 10^{10}$ viral particles dose group. 244 (96%, 95% CI 93–98) of 253 participants in the $1 \times 10^{11}$ viral particles dose group and 125 (97%, 92–99) of 129 participants in the $5 \times 10^{10}$ viral particles dose group showed seroconversion of RBD-specific ELISA antibodies at day 28, whereas the participants in the placebo group showed no antibody increase from baseline.

Both vaccine doses induced significant neutralising antibody responses to live SARS-CoV-2, with GMTs of 19·5 (95% CI 16·8–22·7) and 18·3 (14·4–23·3) in participants in the $1 \times 10^{11}$ and $5 \times 10^{10}$ viral particles dose groups, respectively, at day 28 post vaccination (figure 2). Seroconversion of the neutralising antibody responses to live SARS-CoV-2 occurred in 148 (59%, 95% CI 52–65) of 253 participants receiving the $1 \times 10^{11}$ viral particles dose, and in 61 (47%, 39–56) of 129 participants receiving the $5 \times 10^{10}$ viral particles dose 28 days post vaccination. The GMTs of neutralising antibody responses to pseudovirus were 61·4 (95% CI 53·0–71·0) in the $1 \times 10^{11}$ viral particles dose group and 55·3 (45·3–67·5) in the $5 \times 10^{10}$ viral particles dose group, with seroconversion of 214 (85%, 95% CI 80–89) and 107 (83%, 76–88), respectively. No significant differences were observed between the two dose groups in the neutralising antibody responses to live virus and pseudovirus.

Before vaccination, 266 (52%) of 508 participants had high pre-existing anti-Ad5 neutralising antibodies (table 1). Participants with low pre-existing anti-Ad5 immunity had
RBD-specific ELISA antibody and neutralising antibody levels that were approximately two-times higher than the participants with high pre-existing anti-Ad5 immunity (appendix pp 2–3). Increasing age was found to be another independent negative impact factor on the RBD-specific ELISA antibody (p=0·0018), and neutralising antibody responses to live virus (p=0·0001) or pseudovirus (p=0·046; appendix pp 4–6). The stratified analysis based on age found that participants aged 55 years or older were associated with relative low antibody responses in both dose groups post vaccination, particularly in terms of neutralising antibodies to live virus (appendix pp 7–8). Nevertheless, the ELISA antibodies to RBD and neutralising antibodies at day 28 in vaccine recipients were still significantly higher than in placebo recipients in this population. Male and female participants who received the vaccine showed similar RBD-specific ELISA antibody and neutralising antibody responses post vaccination (appendix pp 9–10). Both the ELISA antibody titres to RBD and neutralising antibody titres to pseudovirus were significantly correlated with the neutralising antibody titres to live virus, with a correlation coefficient of 0·75, and 0·72 (p<0·0001), respectively.

Baseline ELISpot T-cell responses were negative in 506 (>99%) of 508 participants. Ad5-vectored COVID-19 vaccine induced significant SARS-CoV-2 spike glycoprotein-specific IFNγ-ELISpot responses in 227 (90%, 95% CI 85–93) of 253 participants receiving the 1×10¹¹ viral particles dose, and 113 (88%, 81–92) of 129 participants receiving the 5×10¹⁰ viral particles dose at day 28 (figure 3). A median of 11·0 spot-forming cells (IQR 5·0–25·0) and 10·0 spot-forming cells (6·0–21·0) per 1×10⁵ peripheral blood mononuclear cells in participants in the 1×10¹¹ viral particles and 5×10¹⁰ viral particles dose groups, respectively, were observed at day 28, with increases of more than ten-times in both dose groups. The IFNγ-ELISpot responses were not significantly different between the dose groups at day 28. No positive IFNγ-ELISpot T-cell responses were detected in the placebo group post vaccination. Significant increases of post-vaccination T-cell responses in terms of spot-forming cells were observed both in participants with high and low pre-existing neutralising antibody at day 28. In the participants with high pre-existing immunity against Ad5, 88% of participants across both groups (111 of 126 in the 1×10¹¹ viral particles dose group, and 66 of 75 in the 5×10¹⁰ viral particles dose group) showed positive IFNγ-ELISpot T-cell responses post vaccination. Sex and age of the participants did not differ their IFNγ-ELISpot T-cell responses induced by vaccination (appendix p 11). In addition, 241 (95%, 95% CI 92–97) of 253 participants in the 1×10¹¹ viral particles dose group, and 118 (91%, 85–95) of 129 participants in the 5×10¹⁰ viral particles dose group showed either positive IFNγ-ELISpot T-cell response or seroconversion of neutralising antibody to live SARS-CoV-2 at day 28 post vaccination (appendix p 12).

Within 14 days after the vaccination, 183 (72%) of 253 participants in the 1×10¹¹ viral particles dose group, and 96 (74%) of 129 participants in the 5×10¹⁰ viral particles dose group reported at least one solicited adverse reaction, both of which were significantly higher than the 46 (37%) of 126 participants in the placebo group (p<0·0001; table 2). The most common systemic solicited reactions in the 5×10¹⁰ and 1×10¹¹ viral particles dose groups were fatigue, reported by 42% and 34%; fever, reported by 32% and 16%; and headache, reported by
29% and 28%, respectively. The most common injection site solicited reaction was pain, reported by 57% of the 1×10¹¹ viral particles dose group and 56% of the 5×10¹⁰ viral particles dose group. Although most adverse reactions were reported as either mild or moderate, 24 (9%) of 253 participants receiving the vaccine at 1×10¹¹ viral particles had severe (grade 3) adverse reactions, which was significantly higher than those receiving the vaccine at 5×10¹⁰ viral particles (p=0·0011) or placebo (p=0·0004). The most commonly reported grade 3 adverse reactions was fever, in 20 (8%) of 253 participants in the 1×10¹¹ viral particles dose group, and one (1%) of 129 participants in the 5×10¹⁰ viral particles dose group. High pre-existing Ad5 immunity, increasing age, and male sex were associated with significantly lower occurrence of fever post vaccination (appendix p 13). The grade 3 reactions were self-limited and resolved within 72–96 h without medication (appendix p 13). The unsolicited adverse reactions, which was significantly higher than those receiving the vaccine at 5×10¹⁰ viral particles (p=0·0008). †Multiple comparisons of the dose groups 1×10¹⁰ vp versus 5×10¹⁰ vp; p=0·0038. The p value was generated by comparisons across the three treatment groups. vp=viral particles. *Multiple comparisons of the dose groups 1×10¹⁰ vp versus 5×10¹⁰ vp; p=0·0008. †Multiple comparisons of the dose groups 1×10¹⁰ vp versus 5×10¹⁰ vp; p=0·0003.

**Discussion**

This study is the first randomised controlled trial for evaluation of the immunogenicity and safety of a candidate non-replicating Ad5-vectored COVID-19 vaccine. The phase 2 trial is a necessary and crucial step to turn an early-stage experimental vaccine into a promising vaccine candidate in an efficacy trial in a large population. In this study, a single injection of the Ad5-vectored COVID-19 vaccine at 1×10¹¹ viral particles and 5×10¹⁰ viral particles induced comparable specific immune responses to the spike glycoprotein at day 28, with no significant differences noted between the two groups. The vaccine induced seroconversion of the neutralising antibodies in 59% and 47% of participants, and seroconversion of binding antibody in 96% and 97% of participants, in the 1×10¹¹ and 5×10¹⁰ viral particles dose groups, respectively. Positive specific T-cell responses measured by IFNγ-ELISpot were found in 90% and 88% of participants receiving the vaccine at 1×10¹¹ and 5×10¹⁰ viral particles, respectively. 95% of participants in the 1×10¹¹ viral particles dose group and 91% of the recipients in the 5×10¹⁰ viral particles dose group showed either cellular or humoral immune responses at day 28 post vaccination (appendix p 12). Pre-existing immunity to the Ad5 vector and increasing age could partially hamper the specific immune responses to vaccination, particularly for the humoral immune responses.

| Solicited adverse reactions within 14 days | Vaccine at 1×10¹¹ vp (n=253) | Vaccine at 5×10¹⁰ vp (n=129) | Placebo (n=126) | p value |
|----------------------------------------|-------------------------------|-------------------------------|-----------------|---------|
| Any                                    | 183 (72%)                     | 96 (74%)                      | 46 (37%)        | <0·0001 |
| Grade 3                                | 24 (9%)                       | 1 (1%)                        | 0               | <0·0001 |
| Injection site adverse reactions       |                               |                               |                 |         |
| Pain                                   | 145 (57%)                     | 72 (56%)                      | 11 (9%)         | <0·0001 |
| Induration                             | 12 (5%)                       | 2 (2%)                        | 0               | 0·014   |
| Grade 3 induration                     | 2 (1%)                        | 0                             | 0               | 0·75    |
| Redness                                | 5 (2%)                        | 1 (1%)                        | 2 (2%)          | 0·81    |
| Swelling                               | 10 (4%)                       | 5 (4%)                        | 0               | 0·049   |
| Grade 3 swelling                       | 1 (<1%)                       | 0                             | 0               | 1·0     |
| Itch                                   | 14 (6%)                       | 3 (2%)                        | 0               | 0·0075  |

| Systemic adverse reactions             |                               |                               |                 |         |
|----------------------------------------|-------------------------------|-------------------------------|-----------------|---------|
| Fever (all grades)                     | 82 (32%)                      | 21 (16%)                      | 12 (10%)        | <0·0001 |
| Grade 3 fever                          | 20 (8%)                       | 1 (1%)                        | 0               | 0·0001  |
| Headache                               | 73 (29%)                      | 36 (28%)                      | 17 (13%)        | 0·0031  |
| Grade 3 headache                       | 2 (1%)                        | 0                             | 0               | 0·75    |
| Fatigue                                | 106 (42%)                     | 44 (34%)                      | 21 (17%)        | <0·0001 |
| Grade 3 fatigue                        | 1 (<1%)                       | 0                             | 0               | 1·0     |
| Vomiting                               | 4 (2%)                        | 1 (1%)                        | 1 (1%)          | 0·88    |
| Diarrhoea                              | 19 (8%)                       | 10 (8%)                       | 4 (3%)          | 0·22    |
| Muscle pain                            | 39 (15%)                      | 23 (18%)                      | 3 (2%)          | 0·0002  |
| Grade 3 muscle pain                    | 1 (<1%)                       | 0                             | 0               | 1·0     |
| Joint pain                             | 34 (13%)                      | 13 (10%)                      | 4 (3%)          | 0·0074  |
| Grade 3 joint pain                     | 1 (<1%)                       | 0                             | 0               | 1·0     |
| Oropharyngeal pain                     | 22 (9%)                       | 7 (5%)                        | 6 (5%)          | 0·27    |
| Cough                                  | 12 (5%)                       | 2 (2%)                        | 3 (2%)          | 0·24    |
| Nausea                                 | 20 (8%)                       | 6 (5%)                        | 4 (3%)          | 0·14    |
| Hypersensitivity                       | 0                             | 0                             | 2 (2%)          | 0·061   |
| Dyspnoea                               | 1 (<1%)                       | 0                             | 0               | 1·0     |
| Grade 3 dyspnoea                       | 1 (<1%)                       | 0                             | 0               | 1·0     |
| Appetite impaired                      | 27 (11%)                      | 7 (5%)                        | 3 (2%)          | 0·0089  |
| Syncope                                | 1 (<1%)                       | 1 (1%)                        | 0               | 1·0     |
| Mucosal abnormality                    | 2 (1%)                        | 2 (2%)                        | 2 (2%)          | 0·65    |
| Pruritus                               | 6 (2%)                        | 4 (3%)                        | 6 (5%)          | 0·40    |

| Unsolicited adverse reactions within 14 days | Vaccine at 1×10¹¹ vp (n=253) | Vaccine at 5×10¹⁰ vp (n=129) | Placebo (n=126) | p value |
|---------------------------------------------|-------------------------------|-------------------------------|-----------------|---------|
| Any                                         | 19 (8%)                       | 7 (5%)                        | 7 (6%)          | 0·65    |
| Grade 3                                     | 1 (<1%)                       | 0                             | 0               | 1·0     |

| Overall adverse events within 28 days       | Vaccine at 1×10¹¹ vp (n=253) | Vaccine at 5×10¹⁰ vp (n=129) | Placebo (n=126) | p value |
|---------------------------------------------|-------------------------------|-------------------------------|-----------------|---------|
| Any                                         | 196 (77%)                     | 98 (76%)                      | 61 (48%)        | <0·0001 |
| Grade 3                                     | 24 (9%)                       | 1 (1%)                        | 2 (2%)          | 0·0002  |

Data are number of participants (%). Any refers to all the participants with any grade adverse reactions or events. Adverse reactions and events were graded according to the scale issued by the China State Food and Drug Administration. Grade 3 is severe (ie, prevented activity). The p value was generated by comparisons across the three treatment groups. vp=viral particles. *Multiple comparisons of the dose groups 1×10¹⁰ vp versus 5×10¹⁰ vp; p=0·0008. †Multiple comparisons of the dose groups 1×10¹⁰ vp versus 5×10¹⁰ vp; p=0·0038.
In this study, most reactions reported post vaccination were mild or moderate. Although the proportions of participants who had adverse reactions such as fever, fatigue, and injection site pain were significantly higher in vaccine recipients than those in placebo recipients, adverse reactions within 28 days were generally not severe, and resolved within a short period of time (no more than 48 h). Among recipients of either dose of the Ad5-vectored COVID-19 vaccines, all grade 3 adverse reactions were reported from the dose group of 1×10¹¹ viral particles, with the exception of one from the 5×10¹⁰ viral particles dose group. Although this study was powered only to capture common adverse events after immunisation, the results suggest that the experimental Ad5-vectored COVID-19 vaccine has a good safety profile, which was in line with the results of our phase 1 trial in healthy adults.

We initiated this phase 2 trial before the full analysis of the data from the phase 1 study was available. The vaccine doses chosen were mainly based on the safety data from the dose-escalating phase 1 trial: a higher proportion of participants reported grade 3 adverse reactions in the high-dose group (1·5×10¹¹ viral particles) compared with low-dose (5×10¹⁰ viral particles) or middle-dose (1×10¹¹ viral particles) groups (17% vs 6% and 6%, respectively). Therefore, we assumed that the vaccine doses at 1×10¹¹ and 5×10¹⁰ viral particles might have similar safety profiles. In addition, an increasing antigen dose is often associated with increasing immunogenicity; thus, we expected the vaccine dose at 1×10¹¹ viral particles to be better for the two doses. Therefore, we designed the randomisation of this study in a ratio of 2:1:1 for the dose groups of 1×10¹¹ and 5×10¹⁰ viral particles, and placebo group, respectively, giving more weight to the first group.

We found, in contrast to our expectations, that the vaccine at 5×10¹⁰ viral particles had a better safety profile than, and comparable immunogenicity to, the vaccine at 1×10¹¹ viral particles.

Age and pre-existing anti-Ad5 immunity of the participants could have affected the candidate vaccine’s safety and immunogenicity. We noted that the occurrence of fever was associated with decreasing age and low pre-existing immunity to the vaccine vector Ad5 virus. 19 (90%) of the 21 participants who experienced grade 3 fever had no pre-existing immunity to Ad5, with a neutralising antibody titre below detection. Increasing age and high pre-existing anti-Ad5 immunity were found to be able to significantly reduce the immune responses to the vaccine. In some participants with high pre-existing anti-Ad5 immunity, one injection of the vaccine might be inadequate to induce a high level of humoral immune responses, particularly for people aged 55 years or older. These results align with the finding that older people are more likely to have exposure history to Ad5, with higher baseline neutralising antibody to Ad5, which indicates that this population might be more tolerant of higher dose or a booster dose regimen of the Ad5-vectored COVID-19 vaccine than people who are young and naive to Ad5. Pre-existing anti-Ad5 immunity is considered to be the biggest obstacle for the candidate Ad5-vectored COVID-19 vaccine to overcome. A flexible additional dose (between months 3 and 6) might be a potential solution to provide enhancement of immune responses, according to our previous experience with an Ad5 vector-based Ebola vaccine in a homologous prime-boost immunisation study. More evidence about the immunogenicity and feasibility of additional dose immunisation in the older population will be evaluated in a phase 2b trial. However, the vaccine recipients in this study showed increased anti-Ad5 neutralising antibodies, by 5·0-times and 3·8-times in the 1×10¹¹ viral particles and 5×10¹⁰ viral particles dose groups, respectively, at day 28 post vaccination (appendix p 17). The high level of anti-Ad5 immunity could affect the boosting effect of the vaccine; therefore, we planned to follow the dynamic change of the Ad5-specific antibodies in participants until month 6 to determine the timing of booster administration.

We determined the participants’ serostatus before and after immunisation by ELISA, neutralising tests to live SARS-CoV-2 or pseudovirus, and ELISpot, providing evidence of humoral and cell-mediated immunity for the candidate vaccine. Because the live virus neutralising antibody test needs to be done in biosafety level three laboratories, a pseudovirus neutralising antibody test was developed to serve as an alternative. However, we found the magnitudes of neutralising antibody responses to pseudovirus were greater than those to the live virus, which might be associated with the different methodological principles of the two tests. In the pseudovirus neutralising test, when the specific antibody in serum binds to the pseudovirus, it inhibits the pseudovirus from entering the cells, reducing the expression of luciferase on the cell surface. Thus, we can calculate the amount of neutralising antibodies to pseudovirus by detecting the total fluorescence. The neutralising antibody is detected by measuring the cytopathic effect after the viral infection. Although, generally, the output values of the two methods are correlated, the two methods have different detection sensitivities and the detection values do not always have a one-to-one corresponding relationship.

Both neutralising antibody and T-cell responses were important in eliminating the virus and controlling disease development in patients with COVID-19 who were naturally infected by SARS-CoV-2. Antibodies are very likely to be effective against SARS-CoV-2, considering that convalescent serum samples have been applied with apparently good clinical results in COVID-19. But for the vaccine-induced immune responses, whether neutralising antibody alone is capable of preventing infection remains undetermined. Specific T-cell responses are essential for directly attacking and killing virus-infected cells. In addition, the CD4 T-cell responses are critical for the
cytotoxic T-cell response and the maturing of neutralising antibodies. Thus, the evaluation of the cell-mediated responses, in addition to the neutralising antibodies, is important for a successful candidate vaccine.

Our trial has some limitations. First, this phase 2 trial started before the full analysis of the data from the phase 1 study was available, so we did not calculate the sample size based on study power in advance, which might lead to a lack of power to show the difference between dose groups. Second, the participants included in this study are all from Wuhan, China. The baseline anti-Ad5 immunity of the participants seemed to be representative of Chinese adults, according to the previously reported studies; however, anti-Ad5 immunity in adults varies from place to place globally, with reported proportions of adults with neutralising antibodies titres for Ad5 of more than 1:200 of about 80% in India, 78% in Kenya, 67% in Thailand, 64% in Uganda, around 60% in South Africa, 45% in Sierra Leone, and less than 30% in the USA.

We might expect the candidate Ad5-vectored vaccine to have a superior immunogenicity in the population with a lower pre-existing anti-Ad5 immunity than observed in this phase 2 trial. Third, this trial did not include children. Although COVID-19 appears to have a more benign course in children, with almost no fatalities reported, an ideal candidate vaccine for the ongoing pandemic should cover susceptible populations in all ages. Fourth, we report only data within 28 days of vaccination, and do not include data about the durability of the vaccine-induced immunity, which was not available at the time of publication. A subset of patients infected with SARS-CoV-2 might not develop long-lasting antibodies to the virus, and S-antibodies were reported to rapidly decline for people infected with seasonal coronaviruses and who recovered from COVID-19, especially those with mild symptoms or asymptomatic infection. The ongoing phase 1 and 2 trials will enable the continued collection of safety data and assessment of antibody persistence over a 6-month period. Fifth, in this study, no participants had SARS-CoV-2 exposure after the vaccination, so we were unable to assess the efficacy of the candidate vaccine or any immunological risk associated with antibody induced by vaccination when exposed to the virus. However, the risk of COVID-19 and antibody-enhanced disease on exposure to the virus will be monitored long term. Finally, the clinical significance of these changes is difficult to assess because of the absence of an identified correlate of protective immunity, and reference standards for measuring neutralising antibodies against COVID-19. Future studies will need to establish an immune correlate of protection and a protective threshold to assess the feasibility of using the Ad5-vectored COVID-19 vaccine to provide protection for high-risk populations or for outbreak intervention.

WHO is facilitating collaboration and efforts to support vaccine development by defining the desired characteristics of promising candidate vaccines to combat COVID-19. This situation emphasises the importance of being prepared to initiate an international multicentre, randomised, double-blind, controlled phase 3 effectiveness trial as soon as possible. One immunisation of the Ad5-vectored COVID-19 vaccine at 5×10⁹ viral particles has a good safety profile (limited to common adverse reactions following immunisation) and could elicit significant specific immune responses to SARS-CoV-2, making it a potential candidate for emergency vaccination of acute protective response.

In conclusion, the results of this trial have extended our knowledge of the immunogenicity and safety of the Ad5-vectored COVID-19 vaccines. The results support testing of the Ad5-vectored COVID-19 vaccine at 5×10⁹ viral particles in a phase 3 effectiveness trial in healthy adults.
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