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## **Immunogenicity and Safety of a SARS-CoV-2 Inactivated Vaccine in Healthy Adults Aged 18-59 years: Report of the Randomized, Double-blind, and Placebo-controlled Phase 2 Clinical Trial — [Source link](#)**

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**Topics:** Inactivated vaccine, Immunogenicity, Vaccination and Adverse effect

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1 **Full title**

2 **Immunogenicity and Safety of a SARS-CoV-2 Inactivated Vaccine in Healthy**  
3 **Adults Aged 18-59 years: Report of the Randomized, Double-blind, and**  
4 **Placebo-controlled Phase 2 Clinical Trial**

5 **Running title**

6 Phase 2 Clinical Trial of SARS-CoV-2 Inactivated Vaccine

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69 **Footnote**

70 \*The first six authors, YZ, GZ, HP, CL, BK, and YH, contribute equally to the

71 manuscript and are listed as the first authors.

72 ¶The last five authors, JL, XW, MX, QG, and FZ, contribute equally to the

73 correspondence and are listed as the corresponding authors.

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78 **ABSTRACT**

79 **BACKGROUND**

80 The top priority for the control of COVID-19 pandemic currently is the development  
81 of a vaccine. A phase 2 trial conducted to further evaluate the immunogenicity and  
82 safety of a SARS-CoV-2 inactivated vaccine (CoronaVac).

83 **METHODS**

84 We conducted a randomized, double-blind, placebo-controlled trial to evaluate the  
85 optimal dose, immunogenicity and safety of the CoronaVac. A total of 600 healthy  
86 adults aged 18-59 years were randomly assigned to receive 2 injections of the trial  
87 vaccine at a dose of 3 µg/0.5 mL or 6 µg /0.5mL, or placebo on Day 0,14 schedule or  
88 Day 0,28 schedule. For safety evaluation, solicited and unsolicited adverse events  
89 were collected after each vaccination within 7 days and 28 days, respectively. Blood  
90 samples were taken for antibody assay.

91 **RESULTS**

92 CoronaVac was well tolerated, and no dose-related safety concerns were observed.  
93 Most of the adverse reactions fell in the solicited category and were mild in severity.  
94 Pain at injection site was the most frequently reported symptoms. No Grade 3 adverse  
95 reaction or vaccine related SAEs were reported. CoronaVac showed good  
96 immunogenicity with the lower 3 µg dose eliciting 92.4% seroconversion under Day  
97 0,14 schedule and 97.4% under Day 0,28 schedule. 28 days after two-dose  
98 vaccination, the Nab levels of individual schedules range from 23.8 to 65.4 among  
99 different dosage and vaccination schedules.

100 CONCLUSIONS

101 Favorable safety and immunogenicity of CoronaVac was demonstrated on both  
102 schedules and both dosages, which support the conduction of phase 3 trial with  
103 optimum schedule/dosage per different scenarios.

104 **Keywords:** COVID-19; SARS-CoV-2; Inactivated vaccine; Clinical Trial.

## 105 **BACKGROUND**

106 In January 2020, outbreaks of coronavirus disease in 2019 (COVID-19) caused by  
107 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) escalated rapidly,  
108 and since then COVID-19 cases have been reported in over 200 countries and  
109 territories. The pandemic continues to spread unabated affecting the health and  
110 changing the lifestyles of people globally.<sup>1</sup> To reduce the disease burden and stop the  
111 community-wide transmission of COVID-19 across the globe, specific therapeutic  
112 agents or vaccines are urgently needed. Till now, more than 120 vaccine candidates  
113 have been reported to be under development and at least 23 have progressed to the  
114 clinical evaluation stage.<sup>2</sup>

115 The inactivated SARS-CoV-2 vaccine with aluminum hydroxide developed by  
116 Sinovac Life Sciences Co., Ltd., also known as CoronaVac, has been shown to be safe  
117 and could induce SARS-CoV-2 specific neutralizing antibodies in mice, rats, and  
118 nonhuman primates.<sup>3</sup> On the basis of the results obtained from our phase 1 trial, no  
119 safety concerns have been identified. Notably, immunization of CoronaVac induced  
120 immune responses against SARS-CoV-2 in adults. Here, we report the results of the  
121 phase 2 trial.

## 122 **METHODS**

### 123 **TRIAL DESIGN AND OVERSIGHT**

124 This double-blind, randomized and placebo-controlled phase 2 clinical trial based on a  
125 seamless design was registered at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT04352608) and was  
126 conducted in Suining County, Jiangsu Province, China. Detailed information about  
127 the trial has been provided in our previous phase 1 study. The trial protocol and the



128 informed-consent form were approved by the ethics committee of the Jiangsu  
129 Provincial Center for Disease Control and Prevention (JSCDC). This clinical trial was  
130 conducted in accordance with the Chinese regulatory requirements and the standards  
131 of good clinical practice.

132 Before enrollment, written informed consent was obtained from each participant. The  
133 main exclusion criteria included high-risk epidemiological history, positive IgG, IgM  
134 or nucleic acid test of pharyngeal or anal swab, axillary temperature  $>37.0^{\circ}\text{C}$ , allergy  
135 to a vaccine component, and other unsuitable conditions.

136 A total of 600 healthy adults aged 18-59 years were randomly assigned into 3 groups  
137 in a ratio of 2:2:1 to receive 2 injections of the trial vaccine at a dose of 3  $\mu\text{g}/0.5\text{ mL}$   
138 or 6  $\mu\text{g}/0.5\text{ mL}$ , or placebo on a Day 0,14 schedule or a Day 0,28 schedule, according  
139 to a random list generated by an independent statistician..

#### 140 **VACCINE**

141 The vaccine candidate was an inactivated SARS-CoV-2 whole virion vaccine with  
142 aluminium hydroxide as adjuvant (CoronaVac) developed by Sinovac Life Sciences  
143 Co., Ltd. SARS-CoV-2 virus was propagated in Vero cells and harvested. The  
144 harvested virus was inactivated using  $\beta$ -propiolactone and further purified. The bulk  
145 vaccine material obtained from this step was then adsorbed onto aluminium hydroxide  
146 and formulated with phosphate-buffered saline (PBS) and sodium chloride as  
147 inactivated final product. The dosage of 3  $\mu\text{g}/0.5\text{ mL}$  and 6  $\mu\text{g}/0.5\text{ mL}$  were adopted in  
148 this study. Whereas the placebo contained aluminum hydroxide diluents with no  
149 antigen. Both were administered intramuscularly on the schedule of Day 0,14 or Day  
150 0,28.

151 **SAFETY ASSESSMENT**

152 For safety evaluation of CoronaVac, the participants who received at least one dose of  
153 vaccination was included. All vaccinated subjects were observed for immediate  
154 adverse events (AEs) on-site for at least 30 minutes after each administration. Diary  
155 cards were issued to the participants to record the solicited AEs (e.g. pain, induration,  
156 swelling, redness, rash, pruritus) occurring on day 0~7 and unsolicited AEs (e.g. fever,  
157 acute allergic reaction, skin and mucosa abnormality, diarrhea, anorexia, vomiting,  
158 nausea, muscle pain, headache, cough, fatigue) occurring on day 0~28. Data on  
159 serious adverse events (SAEs) were collected throughout the trial. All AEs were  
160 assessed for severity, and the relationship to vaccination was decided by investigators  
161 before unblinding.

162 **IMMUNOGENICITY**

163 To assess immune response, blood samples were collected from each participant  
164 different time points (0/28/42<sup>th</sup> day for Day 0,14 schedule, and 0/56<sup>th</sup> day for Day 0,28  
165 schedule). The ability of the antibodies present in the blood sample to bind the  
166 receptor binding domain (RBD) of SARS-CoV-2 was assessed by enzyme-linked  
167 immunosorbent assay (ELISA). A dilution of 1:160 was considered as a positive  
168 cutoff value. We also measured neutralizing antibody titer (Nab) using a modified  
169 cytopathogenic effect assay. A titer of 1:8 or higher indicated seropositivity.  
170 Seroconversion was defined as a change from seronegative (<1:8) to seropositive ( $\geq$   
171 1:8) or a 4-fold increase from baseline titers if seropositive.

172 The neutralizing antibody assay was performed by Chinese National Institutes for  
173 Food and Drug Control, and the ELISA was performed by Sinovac Biotech.

174 **NEGATIVE STAIN**

175 Virus particles of vaccine used for phase 1 and 2 were diluted to a concentration of  
176 0.04 mg/mL, deposited on a glow-discharged carbon-coated copper grid (Electron  
177 Microscopy Sciences) and after 1 min, washed twice with buffer (20 mM Tris, 200  
178 mM NaCl, pH 8.0), and stained with 1% phosphotungstic acid (pH 7.0) for 1 min.  
179 Then the grid was imaged at room temperature using FEI Tecnai Spirit electron  
180 microscope (Thermo Fisher Scientific) operated at an acceleration voltage of 120 kV.

181

182 **STATISITICAL ANALYSIS**

183 Safety evaluation was performed on participants who received at least 1 dose of the  
184 vaccine or placebo by comparing the overall incidence rate of solicited and  
185 unsolicited AEs among relevant groups. Immunogenicity assessment was performed  
186 on the per-protocol set (PPS). The seroconversion rate was defined as a change from  
187 seronegative to seropositive or a 4-fold increase from baseline titers if seropositive.  
188 The titer distributions were described with reverse cumulative distribution curves and  
189 were tested with the nonparametric Kruskal-Wallis test over the groups.

190 The Pearson Chi-square test or Fisher's exact test was adopted for the analysis of  
191 binary outcomes. Clopper-Pearson method was used to compute the 95% confidence  
192 intervals (CIs) of the binary outcome. ANOVA method was utilized to compare the  
193 GMTs among groups. Hypothesis testing was two-sided with an alpha value of 0.05.  
194 Analyses were conducted by SAS 9.4 (SAS Institute, Cary, NC, USA).

195 **RESULTS**

196 **STUDY POPULATION**

197 From 29 April to 5 May 2020, 600 subjects were enrolled and randomly assigned to  
198 receive first of the CoronaVac or placebo dose. All subjects were included into the  
199 safety assessment. During this trial, 297 subjects put on Day 0,14 schedule and 294  
200 subjects following Day 0,28 schedule were included in the per-protocol cohort for  
201 immunogenicity analysis. These subjects received the 2 injections, attended all visits  
202 and gave planned blood sample. Information about study enrollment, randomization,  
203 and vaccination is shown in Fig. S1.

204 Baseline demographic characteristics at enrollment were similar among these groups  
205 in terms of sex, mean age, height, and weight (Table 1).

206

207

208 **Table 1. Baseline Characteristics of the Study Participants.\***

Characteristics	3 µg Group	6 µg Group	Placebo	<i>P</i>
Day 0,14 schedule				
N	120	120	60	
Age (years)	42.0±10.2	42.4±9.0	43.6±7.6	0.5543
Gender (male/female)	54/66	48/72	25/35	0.7305
Height (m)	1.7±0.1	1.6±0.1	1.6±0.1	0.3864
Body weight (kg)	67.8±11.7	68.7±11.5	68.4±10.9	0.8258
BMI (kg/m <sup>2</sup> )	24.9±3.6	25.5±3.2	25.5±3.0	0.2930
Day 0,28 schedule				
N	120	120	60	

Age (years)	41.5±9.6	40.6±9.9	44.3±8.4	0.0472
Gender (male/female)	63/57	63/57	30/30	0.9417
Height (m)	1.7±0.1	1.7±0.1	1.7±0.1	0.9433
Body weight (kg)	70.0±11.8	70.0±12.2	72.1±12.2	0.4704
BMI (kg/m <sup>2</sup> ) §	25.2±3.1	25.2±3.3	26.1±3.1	0.1741

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209 \* Plus-minus values are means ±SD.

210 § BMI=body mass index.

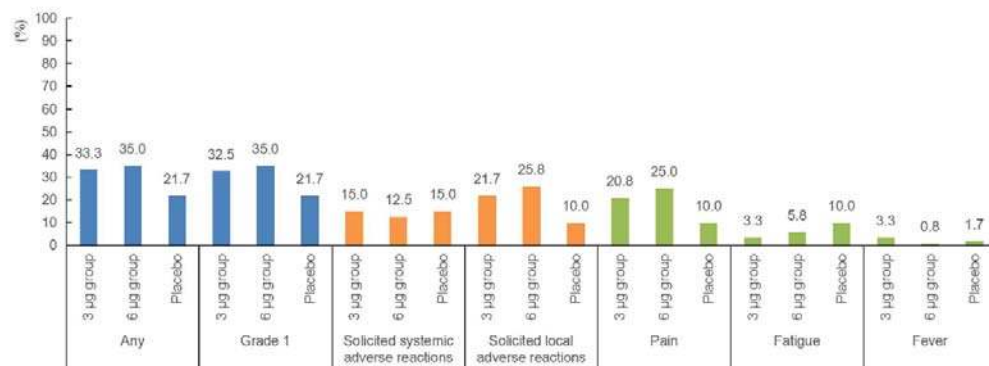
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## 212 **ADVERSE REACTIONS**

213 For subjects in Day 0,14 schedule, the incidence rates of adverse reactions in 6 µg, 3  
214 µg and placebo group were 35.0%, 33.3% and 21.7%, respectively; while the  
215 corresponding incidence rates were 19.2%, 19.2% and 18.3% in Day 0,28 schedule,  
216 respectively. Within each schedule, there was no significant difference in the  
217 occurrence of adverse reactions among all vaccine and placebo groups (Fig. 1). Most  
218 of the adverse reactions were solicited adverse reactions and mild in severity. After  
219 each injection, pain at the injection site was the most frequently reported local  
220 symptoms, which reported in 61 subjects (20.3%) on Day 0,14 schedule and 31  
221 subjects (10.3%) on Day 0, 28 schedule. (Additional detailed results related to adverse  
222 reactions are available in Table S1).

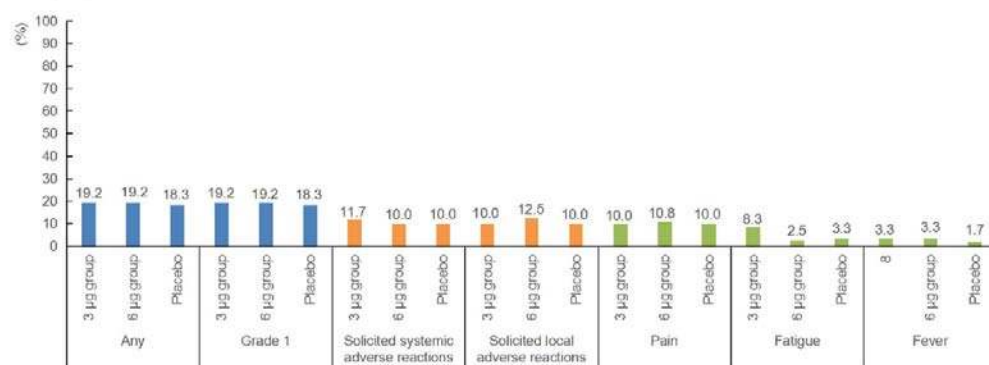
223 We did not observe any Grade 3 adverse reaction. Most reported adverse reactions  
224 resolved within 72 hours after vaccine administration. During the follow-up period, 3  
225 SAEs were reported from 3 subjects and neither was vaccine related.

**A. Day 0,14 Schedule**



226

**B. Day 0,28 Schedule**



227

228 **Figure legends**

229 **Figure 1. Incidence rates of adverse reactions among different groups in phase 2.**

230 (A) The incidence rates of adverse reactions among different groups with a Day 0,14 schedule. (B)

231 The incidence rates of adverse reactions among different groups with a Day 0,28 schedule.

232

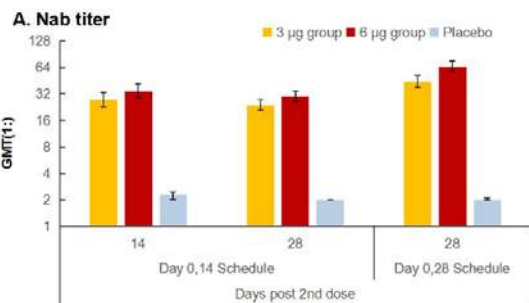
233 **IMMUNOGENICITY**

234 At baseline, all the 600 subjects were seronegative (with Nab titers of <1:8); but the

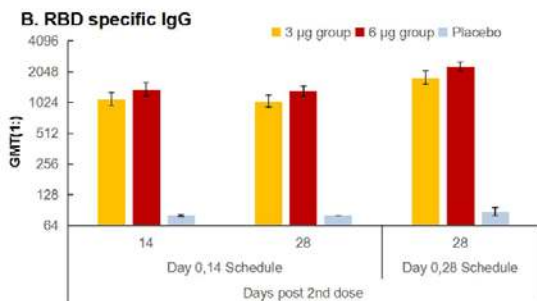
235 seroconversion rates increased over 90% during the later stages of the trial. Within

236 each dosage, there was no significant difference in the seroconversion rates between  
237 Day 0,14 and Day 0,28 schedule. For the antibody response against the receptor  
238 binding domain, similar results were observed (Table S2). No changes in  
239 seropositivity frequencies and GMTs from baseline were found for the placebo group.

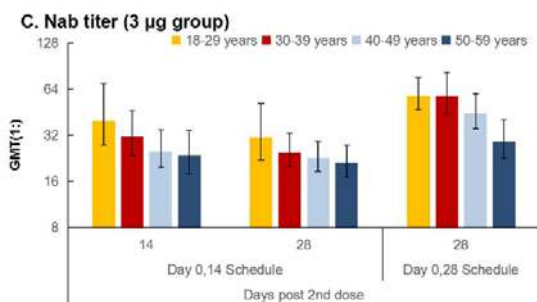
240 For subjects on Day 0,14 schedule, the GMT increased to 34.5 (95% CI, 28.5 to 41.8)  
241 and 27.6 (95% CI, 22.7 to 33.5) in 6 µg and 3 µg group, respectively, and remained  
242 stable after 28 days from the second injection (Fig. 2A). The neutralizing antibody  
243 titers for subjects on Day 0, 28 schedule increased significantly 28 days after the  
244 second injection, when compared to those of subjects on Day 0,14 schedule within  
245 each dosage group. Almost similar trends like those observed for the neutralizing  
246 antibody were observed during the evaluation of the IgG antibody level (Fig. 2B). In  
247 addition, the neutralizing antibody titers significantly decreased with increasing age  
248 (Fig. 2C and 2D); younger subjects tended to have a higher level of neutralizing  
249 antibody titers .



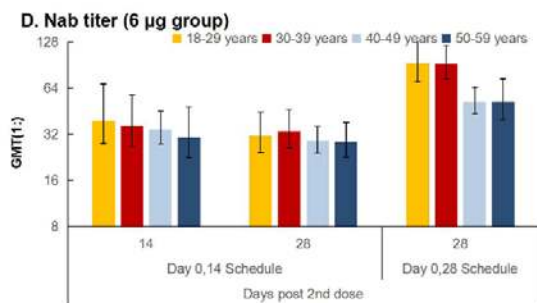
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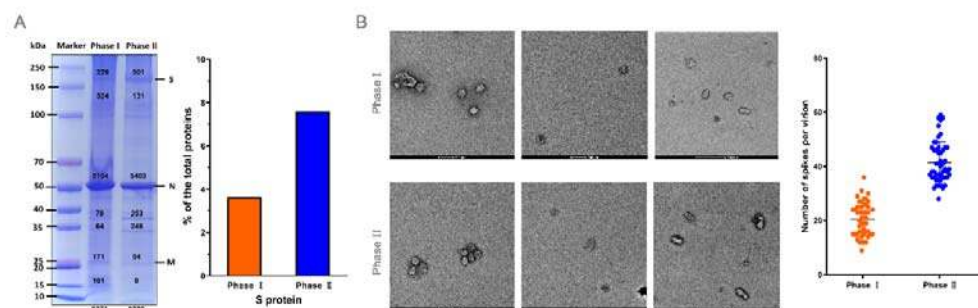
254 **Figure legends**

255 **Figure 2. Antibody Response in the Per-Protocol Cohort.**



256 (A) The neutralizing antibody titer in all participants 14 and 28 days after second dose in Day 0,14  
257 schedule and 28 days after second dose in Day 0,28 schedule. (B) The RBD specific IgG antibody  
258 titer in all participants 14 and 28 days after second dose in Day 0,14 schedule and 28 days after  
259 second dose in Day 0,28 schedule. (C) The neutralizing antibody titer among different age-groups  
260 at different time points from all participants that received 3  $\mu$ g vaccine. (D) The neutralizing  
261 antibody titer among different age-group at different time points from all participants that received  
262 6  $\mu$ g vaccine.

263



264

## 265 Figure legends

### 266 Figure 3. The proportion of Spikes in CoronaVac used for phase 1 and 2 vaccine evaluation.

267 (A) Protein composition analysis of CoronaVac samples from phase I and II by a NuPAGE 4-12%  
268 Bis-Tris gel, followed by whole-gel protein staining using Coomassie Blue gel staining reagent  
269 (45% methanol, 10% glacial acetic acid, 0.25% Coomassie Blue R-250). The viral protein bands  
270 of vaccine strain used for phase I and II were quantified by densitometry using ImageJ software  
271 with values depicted in the gel. The proportions of spikes to the total proteins in each gel lane in  
272 CoronaVac samples used for phase 1 and 2 were calculated separately. (B) Representative  
273 negative staining images of the CoronaVac samples used in phase 1 and 2 trials. Three images  
274 were randomly selected for each phase. Grouped scatter plot showing the numbers of Spikes on  
275 two-dimensional projections of randomly selected 50 virions of CoronaVac samples used for  
276 phase I (left) and phase II (right), respectively.

## 277 **DISCUSSION**

278 This trial demonstrated that the 2 doses of different dosage of CoronaVac were well  
279 tolerated and immunogenic in healthy adults aged 18-59 years. The incidence rates of  
280 adverse reactions in the 6 µg and 3 µg group were comparable, indicating that there  
281 was no dose-related aggravating concern on safety. Furthermore, no SAEs related to  
282 vaccine occurred, and most adverse reactions reported were generally assessed to be  
283 mild. The safety profile of CoronaVac is comparable to that observed in our phase 1  
284 clinical trial [see the coordinated submission], and to other inactivated vaccine  
285 formulations manufactured by Sinovac.<sup>4,5</sup> Compared with other COVID-19 vaccine  
286 candidates, the incidence rate of fever was relatively low in our clinical trial, which  
287 further indicates that CoronaVac was well tolerated.<sup>6-10</sup>

288 It's worth noting that the immune responses elicited in phase 2 were much better than  
289 those recorded in phase 1, with seroconversion rates over 90%. Our preclinical  
290 investigations had revealed that cell culture technology closely correlated with viral  
291 propagation and affected viral morphology, protein composition and prefusion  
292 conformation of spikes.<sup>3</sup> In both preclinical study and phase 1 trials, a 50-liter culture  
293 of Vero cells grown in the Cell Factory system was used, while an optimized process  
294 for growing cells using a highly automated bioreactor, where cell culture parameters  
295 like dissolved oxygen, pH, and CO<sub>2</sub>/O<sub>2</sub> gas levels, were controlled precisely, was  
296 developed for producing the CoronaVac for phase 2 trial. To deduce the reasons  
297 underlying the enhanced protective immune responses observed in phase 2 trial, we  
298 examined the molecular differences between the CoronaVac used in phase 1 and 2  
299 trials. Protein composition analysis of the purified inactivated SARS-CoV-2 virions  
300 indicated that the bioreactor-produced CoronaVac possessed higher redundancy of

301 intact spike protein (~180 kDa) when compared to the Cell Factory-yielded  
302 CoronaVac (Fig. 3A). Quantitative analysis showed that the intact spike protein  
303 accounted for ~7% and ~ 3.7 of total protein mass used in phase 1 and 2 trials,  
304 respectively. Electron microscopic examination of the samples further verified that the  
305 average number of spikes per virion of the viral sample used in phase 2 trial was  
306 almost double to those used in phase 1 trial (Fig. 3B). These observations indicated  
307 that CoronaVac used in phase 2 trial contained more *bona fide* immunogens, which  
308 explains its better protective immune responses, highlighting the importance of  
309 developing an optimum manufacturing process and the integration of  
310 multiple-disciplinary techniques, such as genomics and structural biology to support a  
311 new era of precision vaccinology.

312 After two-dose vaccination, immune responses induced by Day 0,28 schedule was  
313 above the value of Day 0,14 schedule regardless of the dosage of the vaccine, which  
314 was consistent with our anticipation. By using Day 0,14 schedule, antibody response  
315 could be induced within a relatively short time period, and this schedule could be  
316 introduced to an emergency use and is of vital importance to handle COVID-19  
317 pandemic situation. Regarding the Day 0,28 schedule, robust antibody response is  
318 generated and longer persistence could be expected, which supports the need for a  
319 routine use under the low incidence rate of COVID-19.

320 Nabs play an important role in virus clearance and have been considered as a key  
321 immune correlate for protection or treatment against viral diseases. Twenty-eight days  
322 after the two-dose vaccination, the Nab levels of individual schedules range from 23.8  
323 to 65.4 in phase 2, which was lower than those of convalescent patients tested by the  
324 same method in the same laboratory, of which the Nab average level was 163.7.<sup>11</sup> We

325 assume the antibody level could provide satisfying protection against COVID-19  
326 disease based on three reasons. Firstly, most of the surrogate endpoints based on  
327 neutralizing antibodies ranges from 8-24, such as EV71 and Varicella vaccines.<sup>12,13</sup>  
328 Secondly, experience from our preclinical study indicated that the neutralizing  
329 antibody titers of 1:24 elicited in macaques models conferred complete protection  
330 against SARS-CoV-2. Thirdly, several studies revealed that antibody responses  
331 generated from natural infection may decreased significantly, such as SARS-Cov-2,  
332 SARS-CoV and MERS-CoV,<sup>14-16</sup> however, recrudescence of these patients has been  
333 rarely reported, which indicated that the immunological memory might play an  
334 important role of prevention of re-infections.

335 Moreover, one prospective goal of our preclinical study and clinical trials was to  
336 establish a vaccine-induced surrogate of protection. Compared with vaccine inducing  
337 high level antibody, those inducing lower antibody level are more likely to produce  
338 evidence on surrogate of protection. Under above assumptions, the dosage of 3  $\mu$ g  
339 with Day 0,14 or Day 0,28 schedule is adopted in our phase 3 trial.

340 When comparing antibody levels between age-groups, it should be noted that the  
341 neutralizing antibody titers significantly decreased with increasing age. These results  
342 are consistent with epidemiological trends observed in COVID-19 patients; those with  
343 moderate or severe symptoms tend to be elderly.<sup>17</sup> These results suggest that escalated  
344 dosage or extra dose of CoronaVac might be needed in elderly.

345 Several limitations of this trial should be noted. Firstly, we only assessed the humoral  
346 immunity in phase 2 trial, and more evaluation focus on response of Th1 and Th2 is  
347 ongoing. Secondly, we only reported immune response data on healthy adults, and do

348 not include data on more susceptible populations, such as elderly or with comorbidity;  
349 and also the immune persistence is not available yet, which need to be further studied.

350 Thirdly, we didn't compare the neutralizing antibody titers induced by CoronaVac and  
351 convalescent COVID-19 patients in parallel, however, we conducted this detection of  
352 convalescent serum specimens with same procedure performed in this phase 2 trial.

353 In conclusion, favorable safety and immunogenicity of CoronaVac was demonstrated  
354 on both schedules and both dosages in this phase 2 clinical trial, which support the  
355 conduction of phase 3 trial with optimum schedule/dosage per different scenarios.  
356 Currently, our first priority is to evaluate the protective efficacy of the 3 µg dosage  
357 under Day 0,14 schedule. Moreover, Day 0,28 schedule with 3 µg vaccine will also be  
358 adopted in our future phase 3 clinical trials.

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