CT Application(s) Summary Report

- **Protocol title:** Multicenter, Randomized, Double Blind, Parallel Placebo Controlled, Phase III Clinical Trial to Evaluate the Protective Efficacy, Safety and immunogenicity of Inactivated SARS-CoV-2 Vaccines in Healthy Population Aged 18 years old and above.
- **Protocol code number:** CNBG2020003SQ
- **Public Registry Number: NA**
- **Version:** 3.0
- **Date:** 27/07/2020

- **Investigational Medicinal Product being tested:**

Biological ■ **Pharmaceutical** ☐ **Innovative** ☐
Herbal medicine ☐ **Medical device** ☐

- **Sponsor:**
  - China National Biotec Group Company Limited (CNBG)
  - Wuhan Institute of Biological Products Co., Ltd. (WIBP) (A subsidiary institute under CNBG)
  - Beijing Institute of Biological Products Co., Ltd. (BIBP) (A subsidiary institute under CNBG)

- **Indication:** To prevent SARS-CoV-2 caused disease (COVID-19)

- **Investigator's brochure (IB)**
  - By Beijing Institute of Biological Products Co., Ltd and Duan Kai Company: Wuhan Institute of Biological Products Co., Ltd.

  Version: 4.0
  Date: 01/07/2020

- **Name of all Sites:**
  - Katameya Medical Centre
  - Vacsera Healthcare Facility
- **Name of PI(s):**
  - Dr. Mohamed Hassany
  - Dr. Bassem El Sayed

- **EDA approval date:**
  - Initial approval on protocol V3.0 and IB V4.0: 28/03/2022

- **Summary of pre-clinical studies:**
  - WIBP: The inactivated SARS-CoV-2 Vaccine (Vero cell) is prepared by inoculating Verda Reno cells

(Vero cell) with **SARS-CoV-2 WIV04** strain, culturing, harvesting, inactivating, clarifying, concentrating, second inactivating, purifying and adding aluminum hydroxide adjuvant.

- BIBP: The inactivated SARS-CoV-2 Vaccine (Vero cell) is prepared by inoculating Verda Reno cells (Vero cell) with **SARS-CoV-2 HB02** strain, culturing, harvesting, inactivating, clarifying, concentrating, purifying and adding aluminum hydroxide adjuvant.

1. Pharmacology

1.1. Primary pharmacology

The main aim of the pharmacology studies performed to date was to access the immune response elicited by inactivated SARS-CoV-2 Vaccine (adjuvanted) in mice, rats, guinea pigs, rabbits and Rhesus Macaques monkeys. The immune responses were determined in all species by measuring the neutralizing antibody (NtAb) titers, using a plaque reduction neutralization test (PRNT).

1.1.1. Studies on mice

A) First study

Method: Mice used in this study were aged 6 – 8 weeks (18 – 20 g). Mice were divided into four groups, each group included five animals. Group 1 received adjuvant-only in 2 doses on Day (D) 0 and 14, with blood collected on D 0, 14, and 28. Group 2 received adjuvant-only in 3 doses on D 0, 7, and 14, with blood collections on D 0, 14, and 21. Group 3 was administered 200 WU 0.5 ml M-dose vaccine in 2 doses on D 0 and 14, with blood collections on D 0, 14, and 28. Group 4 received 200 WU 0.5 ml in 3 doses on D 0, 7, and 14, with blood collections on D 0, 14, and 21. All animals were dosed via the intraperitoneal (IP) route.

Results: For Group 1, titers were <20 on D 0, 14 and 28. Group 2 showed <20 on D 0, 14 and 21. Group 3 had <20 on D 0 but rose to 83 and 1821 on D 14 and 28 respectively. Group 4 recorded <21 on D 0, increased to 14 on D 14 and 119 on D 21.

Conclusion: Vaccines can induce specific binding antibodies and neutralizing antibodies which differ in intensity according to different immunization schedules. In this experiment, as the immunization interval got longer (from 7 to 14 days), the vaccine stimulated a higher proportion of mice to produce neutralizing antibodies, and these mice produced higher titer of neutralizing antibodies compared to mice administered with adjuvant only.

B) Second study

Method: The mice in this study were weighing 12-14 g. Mice were divided into fifteen groups, each group included ten animals. Groups 1, 2, and 3 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose) respectively, in 1 dose on D 0, with blood collected on D 7, 14, 21 and 28. Groups 4, 5, and 6 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose) respectively, in 1 dose on D 0 and 7, with blood collections on D 14. Groups 7, 8, and 9 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose) respectively, in 1 dose on D 0 and 14, with blood collections on D 21. Groups 10, 11, and 12 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose) respectively, in 1 dose on D 0 and 21, with blood collections on D 28.

Groups 13, 14, and 15 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose) respectively, in 1 dose on D 0, 7 and 14, with blood collections on D 7, 14, 21 and 28. All animals were dosed via the IP injection route.

Results: The immunogenicity of the inactivated SARS-CoV-2 vaccine was evaluated across multiple groups of mice, with geometric mean titers (GMTs) measured at different time points (D 7, 14, 21, and 28). In Groups 1–3, a time-dependent increase in GMT values was observed. In Group 1, GMTs rose from 84 at D 7 to a peak of 630 at D 21, followed by a slight decline to 588 at D 28. Group 2 demonstrated a consistent increase over time, with GMTs rising from 104 at D 7 to 724 at D 28. Similarly, Group 3 showed a progressive increase, reaching 955 at D 28 from an initial value of 119 at D 7. Groups 4–12 were evaluated at single time points. At D 14, GMT values increased across Groups 4–7, ranging from 256 to 588. At D 21, Groups 8–10 showed further increases, with GMTs between 676 and 832. By D 28, Groups 11 and 12 exhibited substantially higher GMTs of 1663 and 1911, respectively. Groups 13–15 demonstrated the highest immune responses overall. Group 13 showed a marked rise in GMT from 84 at D 7 to 4705 at D 28. Group 14 exhibited a similar but more pronounced trend, with GMT increasing from 97 at D 7 to 8192 at D 28. Group 15 also showed strong immunogenicity, with GMT values reaching 3327 by D 21.

Conclusion:

1) The neutralization of antibody potency GMT in mice with one-time, two-time and three-time of immunization was in a dose-dependent manner, and it was shown as positive correlation with time.
2) In the two-time immunization group, the D0 and D21 interval group obtained the highest neutralization antibody potency of 7 days after the last injection compared to D0, D7 and D0, D14. The immunization effect is dose dependent.
3) The immunization effect of three-time immunization program was significantly superior to one- or two-time program.

1.1.2. Studies on rats

A) First study

Method: Sprague Dawley (SD) rats used in this study weighed between 175 - 200 g. Rats were divided into two groups, each group included five animals. Group 1 received adjuvant-only in 3 doses on D 0, 7 and 14, with blood collected on D 0, 7, 14, and 21. Group 2 received M-dose vaccine (0.5ml) in 3 doses on D 0, 7, and 14, with blood collections on D 0, 7, 14, and 21. All animals were dosed using the intramuscular (IM) route.

Results: In the three-dose immunization and aluminum hydroxide adjuvant groups, the neutralizing antibody GMT of the medium dose rat immune serum is much higher than that of the adjuvant-only group.

Conclusion: Compared with the immunogenicity study results of the mouse 0, 7, 14-day immunization regimen, the immune response in rats receiving the same number of injections was greater.

B) Second study

Method: Half male and half female SD rats were randomly taken aged of 66-67 weeks female rats weighing 175-201 g and male rats weighing 193-218 g. Rats were divided into four groups, each group included ten animals. Group 1 received low-dose (2µg/Rat) in 1 dose on D 1 and 8, with blood collected on D 1, 7, and 21. Group 2 received medium-dose (4µg/Rat) in 1 dose on D 1 and 8, with blood collections on D 1, 7, and 21. Group 3 was administered sub-high dose (8µg/Rat) in 1 dose on D 1 and 8, with blood collections on D 1, 7, and 21. Group 4 received high-dose in 2 doses on D 1 and 8, with blood collections on D 1, 7, and 21. All animals were dosed using the IM route.

Results: The immunogenicity of the inactivated SARS-CoV-2 vaccine was evaluated in rats by measuring GMTs at different time points. In Groups 1 and 2, a clear increase in GMT values was observed between D 7 and D 21. In Group 1, GMT increased from 20 at D 7 to 97 at D 21. Similarly, Group 2 showed an increase from 39 at D 7 to 128 at D 21, indicating a time-dependent immune response. Group 3 demonstrated a more extensive dataset across multiple time points. GMT was 42 at D 7 and increased to 219 at D 21. In Group 4, GMT increased from 60 at D 7 to 223 at D 21, further supporting the observed trend of rising antibody titers over time. Overall, the data demonstrate a clear increase in antibody responses between D 7 and D 21 across all evaluated groups, with Group 3 showing consistent and relatively higher GMT values, supporting the induction of a robust humoral immune response in the rat model.

Conclusion:

1) One (1) week after the first administration (before the second administration, D7), the valences of neutralizing antibody in male and female animals of 2, 4, 8 and 16 µg/animal dose groups were 1:4-1:32, 1:32-1:64, 1:32-1:64 and 1:32-1:128 respectively.

2) At the end of recovery period (2 weeks after the second immunization, D21), the valences of neutralizing antibody in male and female animals of 2, 4, 8 and 16 µg/animal dose groups were 1:32-1:512, 1:32-1:256, 1:64-1:512 and 1:64-1:512 respectively.

3) One (1) week after the first administration (D7), the animals of the administration groups were able to develop a certain degree of immunity, which increased significantly at the end of the recovery period (D21), and the antibody valences of animals of 8 and 16 µg/animal dose groups were significantly higher than that of 2 and 4 µg/animal dose groups. With the increase of immune time and immune dose, the valence of neutralizing antibody increased in each group.

1.1.3. Studies on Guinea pig

A) First study

Method: Guinea pigs were divided into two groups, each group included five animals. Group 1 received adjuvant-only in 2 doses on D 0, 7 and 14, with blood collected on D 0, 7, 14, and 21. Group 2 received M-dose vaccine (0.5ml) in 3 doses on D 0, 7, and 14, with blood collections on D 0, 7, 14, and 21. All animals were dosed via the subcutaneous (SC) route.

Results: The level of neutralizing antibody after two doses (14D) and three doses (21D) immunization was significantly different from that after one doses (7D) and two doses (14D) immunization respectively.

Conclusion: With the increase of immunization doses, the titer of neutralizing antibody increased significantly. The serum neutralizing antibody GMT results demonstrated there was an effective boosting effect following three doses of vaccine.

B) Second study

Method: Female guinea pigs were chosen randomly, weighing 200-300 g. Guinea pigs were divided into six groups, each group included ten animals. Groups 1, 2, and 3 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose), respectively in 1 dose on D 0, with blood collected on D 7, 14, and 21. Groups 4, 5, and 6 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose), respectively in 1 dose on D 0, 7, and 14, with blood collections on D 7, 14, and 21. All animals were dosed using the IP injection route.

Results: The immunogenicity of the inactivated SARS-CoV-2 vaccine was evaluated in guinea pigs by measuring NtAb titers expressed as GMTs at D 7, 14, and 21. Overall, the results were generally consistent with those observed in mice and rats, demonstrating a time-dependent increase in antibody responses. Across all groups, NtAb titers increased progressively from D 7 to D 21. In Group 1, GMT values rose from 6 at D 7 to 30 at D 14 and 64 at D 21. Group 2 showed a similar trend, with GMTs increasing from 20 to 42 and reaching 74 at D 21. Group 3 demonstrated a stronger response, with titers increasing from 21 at D 7 to 45 at D 14 and 104 at D 21. Group 4 exhibited a comparable pattern, with GMT values rising from 5 at D 7 to 34 at D 14 and 119 at D 21. Higher responses were observed in Groups 5 and 6 at later time points. Group 5 showed an increase from 20 at D 7 to 42 at D 14, with a marked rise to 294 at D 21. Similarly, Group 6 demonstrated an increase from 21 to 45, reaching 362 at D 21. Overall, the data indicate a consistent and progressive increase in neutralizing antibody titers over time across all groups, with more pronounced responses in Groups 5 and 6, supporting the immunogenicity of the vaccine in the guinea pig model.

Conclusion:

1) The neutralization of antibody potency GMT in guinea pigs with one-time and three-time of immunization was in a dose-dependent manner, and it was shown as positive correlation with time.
2) The GMT levels of three-time immunization group were better than the one-time immunization group in guinea pig.

1.1.4. Studies on rabbits

A) First study

Method: Rabbits were divided into four groups; each group included one animal. Group 1 received adjuvant-only in 2 doses on D 0 and 14, with blood collected on D 0, 14, and 28. Group 2 received adjuvant-only in 3 doses on D 0, 7, and 14, with blood collections on D 0, 7, 14, and 21. Group 3 was administered 200 WU 0.5 ml M-dose vaccine in 2 doses on D 0 and 14, with blood collections on D 0, 14, and 28. Group 4 received 200 WU 0.5 ml in 3 doses on D 0, 7, and 14, with blood collections on D 0, 7, 14, and 21. All animals were dosed via the SC route.

Results: For Group 1, titers were <20 on D 0, 14 and 28. Group 2 showed <20 on D 0, 7, 14 and 21. Group 3 had <20 on D 0 but rose to 33 and 2560 on D 14 and 28, respectively. Group 4 recorded <20 on D 0 and D 7, increased to 113 on D 14, and 2560 on D 21.

Conclusion: With the increase of injection times, the titers of neutralizing antibodies by both immunization schedules increased. The immunogenicity results of rabbits are similar to those of mice. With a longer immunization interval, the vaccine can stimulate the production of higher levels of neutralizing antibodies compared to adjuvant-only treatment.

B) Second study

Method: Female rabbits weighing 1.5-2.0 Kg were randomly chosen. Groups 1, 2, and 3 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose) respectively, in 1 dose on D 0, with blood collected on D 7, 14 and 21. Groups 4, 5, and 6 received low dose (2 µg/dose), medium dose (4 µg/dose), and high dose (8 µg/dose) respectively, in 1 dose on D 0, 7 and 14, with blood collections on D 7, 14 and 21. All animals were dosed via the IP injection route.

Results: The immunogenicity of the inactivated SARS-CoV-2 vaccine was evaluated in rabbits by measuring NtAb titers expressed as GMTs at D 7, 14, and 21. Across all groups, NtAb titers increased progressively from D 7 to D 21. In Group 1, GMT values increased from 8 at D 7 to 24 at D 14 and 37 at D 21. Group 2 showed a similar trend, with GMTs increasing from 12 to 42 and reaching 56 at D 21. Group 3 demonstrated a stronger response, with titers increasing from 37 at D 7 to 84 at D 14 and 111 at D 21. Group 4 exhibited a comparable pattern, with GMT values rising from 8 at D 7 to 32 at D 14 and 84 at D 21. Higher responses were observed in Groups 5 and 6 at later time points. Group 5 showed an increase from 16 at D 7 to 64 at D 14, with a marked rise to 338 at D 21. Similarly, Group 6 demonstrated an increase from 49 to 111, reaching 388 at D 21. Overall, the data indicate a consistent and progressive increase in neutralizing antibody titers over time across all groups, with more pronounced responses in Groups 5 and 6, supporting the immunogenicity of the vaccine in the rabbit model.

Conclusion:

1) The neutralization of antibody potency GMT in rabbits with one-time and three-time of immunization was in a dose-dependent manner, and it was shown as positive correlation with time.
2) The 28-day neutralization antibody potency of the three-time immunization group with high- , medium- and low-dose was significantly higher than that of the one-time immunization group; the neutralization antibody potency GMT levels of three-time immunization group were better than that of one-time immunization group in rabbits.

1.1.5. Studies on Rhesus monkeys

A) First study

Method: Rhesus monkeys were divided into two groups; group 1 included 2 animals and group 2 included 3 animals. Group 1 received adjuvant-only in 2 doses on D 0 and 14, with blood collected on D 0, 7, 14, and 21. Group 2 received M-dose vaccine (0.5 ml) in 2 doses on D 0 and 14, with blood collections on D 0, 7, 14, and 21. All animals were dosed via the IM route.

Results: For Group 1, titers were <20 on D 0, 7, 14, and 21. Group 2 showed <20 on D 0 and increased to 135, 583, and 846 on D 7, 14, and 21, respectively.
Conclusion: The antibody test results showed that the vaccine had good immunogenicity and could stimulate rhesus monkeys to produce high titers of neutralizing antibody.

B) Second study

Method: Rhesus monkeys were divided into two groups; each group included four animals. Groups 1 and received low-dose (2µg/dose) and high dose (8µg/dose) in 1 dose on D 0 and 14, with blood collected on D 7, 14, and 21. Group 2 received medium-dose (4µg/Rat) in 1 dose on D 1 and 8, with blood collections on D 1, 7, 21, 24/26, and 31/33. All rhesus macaques were dosed via the IM injection route.
Results: The immunogenicity of the inactivated SARS-CoV-2 vaccine was evaluated in rhesus macaques by measuring NtAb titers expressed as GMTs at D 7, 14, and 21. Across all groups, NtAb titers increased progressively from D 7 to D 21. In Group 1, GMT values increased from 5 at D 7 to 45 at D 14, 108 at D 21, 215 at D 24/26, and 512 at D 31/33. Group 2 showed a similar trend, with GMTs increasing from 10 to 64 and reaching 860 at D 31/33. Overall, the data indicate a consistent and progressive increase in NtAb titers over time across all groups supporting the immunogenicity of the vaccine in the rhesus monkey model.
Conclusion: The neutralization antibody in the placebo group was negative at 24 days after vaccine immunization, the neutralization antibody GMT at 24 days after immunization in the low-dose group was 1: 215, and it reached l: 512 at 7 days after challenge. The neutralization antibody GMT was 1: 256 and reached 1: 860 at 7 days after challenge.

1.2. Challenge studies

A) First challenge study

Experimental animals: Eight Rhesus monkeys, aged 6 to 8 years old (4 per sex), were assigned to either low or high dose vaccine groups (3 per group) or the control group (2).
Test item: Inactivated SARS-CoV-2 vaccine (200 WU per 0.5 ml) with aluminum hydroxide adjuvant (Alhydrogel®, Croda Denmark).
Immunization schedule: The 200 WU (0.5 ml) and 1000 WU (0.5 ml) vaccine groups were immunized twice (on D 0 and D 14). The control group was immunized with 0.5 ml Alhydrogel® on D 0 and D 14.
Viral challenging immunization route and dosage: The experimental animals were transferred to the level 4 biosafety laboratory following immunization, to continue feeding and observation. All animals in the control group, low dose (200 WU) and high dose (1000 WU) groups were infected with SARS-COV-2 (1 × 106 TCID50) via tracheal intubation 10 days following the last administration of vaccine on D 14. Viral replication in the lungs of all animals was assessed 3 and 6 days following challenge.
Conclusion: The results showed that viral replication was completely inhibited in both vaccine groups. This corresponded to a high level of serum neutralizing antibodies (1: >1000). A high level of viral proliferation was observed in the lungs of the control animals.

The viral infection leads to moderate to severe viral pneumonia and acute alveolar injury in the control group. Under the same infection dose, the lung injury caused by the virus in the experimental animals was mild and was anticipated to fully resolve.

B) Second challenge study

Experimental animals: 10 Rhesus monkeys, aged 3 to 6 years old, were assigned to either low or high dose vaccine groups (4 per group) or the control group (2).

Test item: Inactivated SARS-CoV-2 vaccine with aluminum hydroxide adjuvant (Alhydrogel®, Croda Denmark).

Immunization schedule: The 2 µg (0.5 ml) and 8 µg (0.5 ml) vaccine groups were immunized twice (on D 0 and D 14). The control group was immunized with 0.5 ml of saline on D 0 and 14.

Viral challenging immunization route and dosage: The experimental animals were transferred to the level 3 biosafety laboratory following immunization, to continue feeding and observation. All animals in the control group, low dose (2µg/dose) and high dose (8µg/dose) groups were infected with SARS-COV-2 (1 × 106 TCID50) via tracheal intubation 10 days following the last administration of vaccine on D 14. Viral replication in the lungs of all animals was assessed 7 days following challenge.

Conclusion: The results showed that viral replication was completely inhibited in both vaccine groups. This corresponded to a high level of serum neutralizing antibodies. A high level of viral proliferation was observed in the lungs of the control animals. In the virus-challenging protection experiments, we have demonstrated that two-time immunization with lose dose (2µg/dose) of BIBP-CorV provided the complete protection in macaques against SARS-CoV-2 challenge, and without detectable antibody-dependent enhancement of infection.

1.3. Safety pharmacology

A formal safety pharmacology study has not been conducted but assessment of safety pharmacology parameters were incorporated in the GLP repeat dose toxicology study in rats and monkeys. No vaccine related changes were reported in clinical observations, body weight, organ weights and histopathology (heart, lungs and brain) following IM administration of inactivated SARS-CoV-2 vaccine (0.5 ml and 1.5 ml) in SD rats (administered 3 times over a two-week period) and in cynomolgus monkeys (4 times over a four- week period). Importantly, no vaccine related changes were reported in electrocardiogram and BP in monkeys.

2. Pharmacokinetics and Drug Metabolism in Animals

No pharmacokinetic experiments for inactivated SARS-CoV-2 vaccine have been performed to date. These are generally not required for vaccines because the kinetic properties of antigens do not provide useful information for determining dose recommendations for clinical studies. Three toxicology studies have been performed for the inactivated SARS-CoV-2 vaccine: one single-dose study in rats and two repeat-dose studies, one in rats and one in cynomolgus monkeys. All three studies were performed to GLP compliant principles.

3. Toxicology

Three toxicology studies have been performed for inactivated SARS-CoV-2 vaccine, one single dose study in rats and two repeat-dose studies, one in rats and one in Cynomolgus monkeys. All three studies were performed to GLP compliant principles.

3.1. Single Dose Studies

3.1.1. SD Rats

A) First study

Methods: Twenty SD rats (10 males and 10 females) were randomly divided into 2 groups according to sex, namely negative control group and test group, with 5 males and 5 females in each group.
The animals in the negative control group were given 2 ml sodium chloride injection IM, and the animals in the test group were given 2 ml (4 x 0.5 ml) of inactivated SARS-CoV-2 vaccine.
All animals were continuously observed for signs of acute reactions for at least 4 hours following administration, and then observed once every morning and afternoon for 14 days. The body weight and food intake of animals were measured regularly. At the end of the observation period (D 15), the animals were euthanized, and gross anatomy observations recorded.
Results: During the test, no death or dying was observed in any group of animals, and no obvious abnormal reactions were observed during the clinical observation period. Compared with the same sex negative control group animals, there was no statistical difference in body weight in the test group ($P > 0.05$). No effect of test vaccine administration on animal food intake was found.
Pathological gross anatomy and naked eye observations showed there were no abnormal changes in the main organs and tissues of animals in each group.
Conclusions: Under the experimental conditions, where the inactivated SARS-CoV-2 vaccine was injected IM with 4 doses per rat, no obvious abnormal reactions were observed. The maximum tolerated dose (MTD) in rats is greater than or equal to 4 x 0.5 mL (200 WU) doses per rat.

B) Second study

Methods: Twenty SD rats (10 males and 10 females) were used in the experiment and randomly divided into 2 groups according to sex, namely negative control group and test group, with 5 males and 5 females in each group. The animals in the negative control group were given 1.5 ml sodium chloride injection intramuscularly, and the animals in the test group were given 1.5 ml/3 doses of inactivated SARS-CoV-2 Vaccine (Vero cells) (8μg per dose. During the test period, the acute toxic reaction symptoms of all animals were continuously observed for at least 4 hours after administration, and then observed once every morning and afternoon for 14 days. The body weight and food intake of animals are measured regularly. At the end of the observation period (D15), the animals were euthanized and observed by gross anatomy.
Results: During the test, no death or dying was found in each group of animals, and no obvious abnormal reaction was found in clinical observation. Compared with the same sex negative control group animals in the same period, there was no statistical difference in body weight in the test group (P >

0.05). No effect of drug administration on animal food intake was found. Pathological gross anatomy and naked eye observation showed that there were no abnormal changes in the main organs and tissues of animals in each group.

Conclusions: Under the experimental conditions, where the inactivated SARS-CoV-2 vaccine was injected IM with 3 doses per rat, no obvious abnormal reactions were observed. The MTD in rats is greater than or equal to 3 x 0.5 ml (8 µg) doses per rat.

3.2. Repeat Dose Studies

3.2.1. SD Rats

A) First study

Methods: One hundred and fifty (150) SD rats, half male and half female, were randomly divided into 7 groups according to their body weight. One hundred and twenty (120) rats were assigned to the main test groups (groups 1 to 4), were used for toxicological study (15 male and 15 female/group) with 30 rats in the satellite groups, groups 5 to 7 were used for serum antibody determination (5 male and 5 female/group). During the test, the negative control group animals were given a sodium chloride injection. The adjuvant control group was given adjuvant control with three doses (0.5 ml/dose) per animal, while the low and high dose vaccine groups were given inactivated SARS-CoV-2 vaccine at one dose (0.5 ml) per animal and three doses (0.5 ml/dose) per animal respectively. The route of administration of animals in each group was IM injection, once a week for 2 weeks, a total of 3 times. During the experiment, clinical observations, body weight, food intake, body temperature, ophthalmic examination, clinical pathology, T lymphocyte subsets, serum cytokines, anti-SARS-CoV-2 specific IgG antibody and neutralization antibodies were measured.

Results: During the experiment, there was no death or dying in any group, there were no abnormal reactions related to test vaccine administration observed during in clinical observation. No abnormalities were found in the anatomical observation of animals euthanized 3 days after the last dose. Compared with the negative control group of the same sex in the same period, IL-6 levels increased in animals receiving low and high doses of the test vaccine (a cytokine associated with a response to infections and tissue injury). IL-6 increases were statistically higher in female animals. No abnormal changes related to vaccine administration were found in the cytokines (IL-2, IL-10, TNF-α and IFN-γ) of animals in each group. At the end of the recovery period (2 weeks), no other pathological changes were found except for a local irritation reaction at the injection site in all groups of animals. Under microscopic examination, no apparent toxic pathological changes related to the test vaccine or adjuvant reference substance were found, except near the injection site.

Antibody test results showed that after immunization with the inactivated SARS-CoV-2 vaccine, SD Rats could be induced to produce neutralizing antibodies and binding antibodies (specific IgG antibodies) against SARS-CoV-2.

Conclusion: Inactivated SARS-CoV-2 vaccine administered 3 times over a two-week period in SD rats (low and high doses) does not result in any apparent systemic toxic reaction. No other pathological

changes were found except local irritation around the injection site in each group of animals. SARS-CoV-2 binding and neutralizing antibodies increased significantly, and correlate with dose and booster effects.

B) Second study

Methods: Two hundred rats (100 male and 100 female) were divided into 10 groups according to body weight, of which 150 rats were used for toxicological studies (15 male and15 female/group, group 1 to 5) and 50 rats were used for antibody research (5 male and 5 female/group, group 6 to 10). Ten groups of animals were intramuscularly injected with negative reference (sodium chloride, group 1 and 6), 2, 4, 8 and 16 μg/animal of the inactivated SARS-CoV-2 Vaccine (Vero Cell, group 2 and 7, group 3 and 8, group 4 and 9 and group 2 and 7), respectively. The injection volume for group 2 to 4 and group 7 to 9 was 0.5 ml/animal while the injection volume for group 1, 5, 6, 10 was 1.0 ml/animal. The route of administration of animals in each group was IM injection, once a week, a total of 2 times. The study protocol is summarized in Table 16. The first 10 animals in group 1 to 5 were euthanized on D11, and the rest 5 animals of each sexuality were observed for a recovery period of 2 weeks then were euthanized on D22. All animals found dead in the toxicology group and the satellite group underwent systematic anatomical and histopathological observation. All surviving animals in group 6 to 10 were euthanized after the last blood sample collection without systematic anatomical observation. During the experiment, clinical observations, body weight, food intake, body temperature, ophthalmic examination, clinical pathology, T lymphocyte subsets, serum cytokines, anti- SARS-CoV-2 specific IgG antibody and neutralization antibodies were measured.

Results: During the experiment, a male animal (#2011343) in the satellite group 9 died on D14. Combining the relevant detection indexes and histopathological results of this group of animals, it was considered that the death of this animal may not be related to the injection. No death or dying animals in the other groups were observed; there were no abnormal reactions related to test vaccine administration observed during clinical observation. No abnormalities were found in the anatomical observation of animals euthanized 3 days after the last dose. Compared with the negative control group of the same sex in the same period, no abnormal changes related to vaccine administration were found in the cytokines (IL-2, IL-10, TNF-α, and IFN-γ) of animals in each group. At the end of the recovery period (2 weeks), no other pathological changes were found except for a local irritation reaction at the injection site in all groups of animals. Under microscopic examination, no apparent toxic pathological changes related to the test vaccine or adjuvant reference substance were found, except near the injection site. Antibody test results showed that after immunization with the inactivated SARS-CoV-2 vaccine, SD rats could be induced to produce neutralizing antibodies and binding antibodies (specific IgG antibodies) against SARS-CoV-2.

Conclusion: The inactivated SARS-CoV-2 vaccine administered 2 times over one week period in SD rats does not result in any apparent systemic toxic reaction. No other pathological changes were found except for local irritation around the injection site in each group of animals. SARS-CoV-2 binding and neutralizing antibodies increased significantly, and correlate with dose and booster effects.

3.2.2. Cynomolgus Monkey

A) First study

Method: Forty (20 male and 20 female) cynomolgus monkeys aged 2.6 - 3 years old, weighing between 1.8 and 4 kg, were injected 4 times over 3 weeks. Injections were given intramuscularly on D1, D8, D15, and D29. During the experiment, clinical observations, body weight, food intake, body temperature, ophthalmic examination, clinical pathology, T lymphocyte subsets, serum cytokines, anti- SARS-CoV-2 specific IgG antibody and neutralization antibodies were measured. Animals underwent detailed clinical observation once a week in addition to one day before each administration and one day after each administration. In addition to clinical observations, body weight, body temperature, electrocardiogram and blood pressure were monitored. Ophthalmic examination, clinicopathological examination, immunological index, antibody test, gross and histopathological examinations were also performed.

Results: During the experiment, there was no death or dying of animals in any group, there was no abnormal reaction related to vaccine administration observed during clinical observation. No abnormalities were found during anatomical observation. As was observed in the rat repeat-dose toxicity study, at the end of recovery period (2 weeks), no other pathological changes were found except a local irritation reaction at the injection site in all groups of animals. SARS-CoV-2 binding and neutralizing antibodies increased significantly and correlated to dose and booster effects. Under microscopic examination, no obvious toxic pathological changes related to the test vaccine or adjuvant were found except for changes around the injection area.

Conclusions: Inactivated SARS-CoV-2 vaccine administered 4 times over a four-week period in cynomolgus monkeys (low and high doses), does not result in any apparent systemic toxic reaction. SARS-CoV-2 binding and neutralizing antibodies increased significantly, and correlate to dose and booster effects. No other pathological changes were found except local irritation/muscle damage around the injection site in each group of animals.

B) Second study

Method: Forty (20 male and 20 female) cynomolgus monkeys aged 2.6 - 3 years old, weighing between 1.8 and 4 kg, were injected 4 times over 3 weeks. Injections were given intramuscularly on D1, D8, D15, and D22. During the experiment, clinical observations, body weight, food intake, body temperature, ophthalmic examination, clinical pathology, T lymphocyte subsets, serum cytokines, anti- SARS- CoV-2 specific IgG antibody, and neutralization antibodies were measured. Animals underwent detailed clinical observation once a week in addition to one day before each administration and one day after each administration. In addition to clinical observations, body weight, body temperature, electrocardiogram, and blood pressure were monitored. Ophthalmic examination, clinicopathological examination, immunological index, antibody test, gross and histopathological examinations were also performed.

Results: During the experiment, there was no death or dying of animals in any group; there was no abnormal reaction related to vaccine administration observed during clinical observation. No abnormalities were found during anatomical observation. As was observed in the rat repeat-dose toxicity study, at the end of the recovery period (2 weeks), no other pathological changes were found except a local irritation

reaction at the injection site in all groups of animals. SARS-CoV-2 binding and neutralizing antibodies increased significantly and correlated with dose and booster effects. Under microscopic examination, no obvious toxic pathological changes related to the test vaccine or adjuvant were found except for changes around the injection area.

Conclusions: The cynomolgus monkeys were administered with the inactivated SARS-CoV-2 vaccine once a week for 3 weeks (2μg/dose, 4 μg/dose and 8 μg/dose), which does not result in any apparent systemic toxic reaction. SARS-CoV-2 binding and neutralizing antibodies increased significantly, and correlate to dose and booster effects. No other pathological changes were found except local irritation/muscle damage around the injection site in each group of animals.

3.3. Genotoxicity/ Carcinogenicity studies

As inactivated SARS-CoV-2 vaccine is a biological product, no genotoxicity studies have been performed with this product as appropriate.

3.4. Reproductive and development toxicity

At this stage of development, no reproductive and development toxicity studies have been performed as appropriate. Appropriate contraception will be used in the clinical trial.

3.5. Other toxicity studies

3.5.1. Local injection site reactions:

Local injection site tolerance has been assessed as part of the GLP-compliant repeat dose toxicity study in rats and monkeys.

Vaccine related injection site reactions were reported following IM administration of inactivated SARS-CoV-2 vaccine (0.5 ml and 1.5 ml) in SD rats (administered 3 times over a two-week period) and in cynomolgus monkeys (4 times over a four-week period).

Under microscopic examination, no obvious toxic pathological changes related to the test vaccine or adjuvant were found except for around the injection site. Changes around the injection site occurred in all animals in the adjuvant control group and low and high vaccine dose groups.

Local reactions were characterized by mild to moderate granulomatous inflammation (macrophages). There was no obvious difference in incidence and lesion degree between the adjuvant control and vaccine test groups. Therefore, it was concluded that local site reactions observed microscopically were related to the aluminum adjuvant and not to test vaccine.

3.5.2. Animal allergy test

Methods: Thirty-six Specific Pathogen-Free (SPF) male Hartley guinea pigs were selected and randomly divided into 4 groups with 9 animals in each group, including a high dose vaccine group (0.5 ml), a low dose (0.05 ml) vaccine group, negative control group, and positive control group. They were sensitized by IM injection into their hind limbs on D1, D3, and D5, and sensitized by intravenous injection on D19 and/or D26. Fourteen days after the last sensitization, the first 3 animals in each group were stimulated by intravenous injection of feet (0.1 ml and 1 ml for low and high vaccine groups, respectively), and observed for at least 30 minutes after injection. If all the animals in each dose group of the test vaccine had an

allergic reaction, the remaining animals in each group would be stimulated on the same day. The remaining animals in the negative control group, the positive control group, and the group without allergic reaction were stimulated again 21 days after the last sensitization. During the test, supplementary tests are needed for the samples causing the first allergic reaction.
Results: Under the experimental conditions, each of the guinea pigs was sensitized with inactivated SARS-CoV-2 vaccine by IM injection of 0.1 x dose and 1 x dose, and then stimulated by intravenous injection of 0.2 x dose and 2 x dose, respectively. No systemic active allergic reaction was measured.
Conclusion: The systemic active allergic reaction in guinea pigs was negative.

- **Summary of previous clinical studies:**

As of 27th April 2020, inactivated SARS-CoV-2 vaccine, has been approved by the National Medical Products Administration (NMPA) for clinical trials. As of the 28th of June 2020, the inactivated SARS-CoV-2 vaccine had been administered to 1,120 healthy volunteers aged 18 years and older in Phase I/II clinical trials following regulatory approval for clinical evaluation by NMPA.
Interim unblinded analyses in adults aged 18–59 years showed that a 2-dose regimen demonstrated good safety and tolerability and induced strong immune responses.
In Phase I, a four-fold increase in antibody response was observed 14 days after the second dose, reaching 100% in the evaluated groups under the D 0/28 schedule. In Phase II, under the D 0/14 and D 0/21 schedules, four-fold increases in antibody or neutralizing antibody responses 14 days after the second dose were reported at 97.6% to 100% in the assessed low- and/or medium-dose groups. Overall, these interim findings indicate that the 2-dose inactivated SARS-CoV-2 vaccine regimen was well tolerated and capable of inducing high antibody and neutralizing antibody responses in healthy adults.

- **Protocol:** Multicenter, Randomized, Double Blind, Parallel Placebo Controlled, Phase III Clinical Trial to Evaluate the Protective Efficacy, Safety and immunogenicity of Inactivated SARS-CoV-2 Vaccines in Healthy Population Aged 18 years old and above. **(CNBG2020003SQ)**

Phase: III

Objective(s):

Primary objectives:

1- To evaluate the protective efficacy against COVID-19 of inactivated SARS-CoV-2 Vaccines (Vero Cell) after 2 doses of immunization in healthy subjects aged 18 years and above.

Secondary objectives:

1- To evaluate the safety of inactivated SARS-CoV-2 vaccines in healthy subjects aged 18 years and above.
2- To evaluate the immunogenicity of inactivated SARS-CoV-2 vaccines in healthy subjects aged 18 years and above.
3- To evaluate the protective efficacy against severe COVID-19 and deaths associated with COVID-19 in 14 days after 2-dose immunization.

Exploratory objectives:

1- Explore the anti-SARS-CoV-2 neutralizing antibody protective level in 14 days after 2-dose immunization (immunological surrogate endpoint).
2- Explore the occurrence of antibody-dependent enhancement (ADE) after immunization.

Endpoints:

Primary endpoints:

1- The efficacy against COVID-19 of the inactivated SARS-CoV-2 vaccine (Vero cell) 14 days after 2 doses of immunization in healthy subjects aged 18 years old and above

Secondary endpoints:

1- The efficacy against severe cases of COVID-19 and deaths accompanied by COVID-19 of the inactivated SARS-CoV-2 vaccine (Vero cell) in 14 days after 2 doses of immunization.

Exploratory endpoints:

1- The protective level of anti-SARS-CoV-2 neutralizing antibody in 14 days after 2 doses of immunization.

Rationale:

Coronavirus disease 2019 (COVID-19) is caused by SARS-CoV-2, a novel beta-coronavirus belonging to the Sarbecovirus subgroup that was first identified in December 2019 following an outbreak of pneumonia of unknown etiology characterized by fever, cough, dyspnea, and diffuse pulmonary infiltrates. The virus is an enveloped, single-stranded positive-sense RNA virus measuring 80–120 nm in diameter with a genome of approximately 30,000 bases — the largest among all known RNA viruses. SARS-CoV-2 enters host cells through binding of its spike (S) protein to the human ACE2 receptor with an affinity approximately 20 times greater than that of SARS-CoV, contributing to its exceptionally high transmissibility through respiratory droplets, close contact, and potentially aerosol and feco-oral routes. By June 2020, the virus had infected more than 9.6 million individuals and caused over 486,000 deaths worldwide, exceeding the combined mortality burden of the SARS (2003) and MERS (2012) outbreaks. Despite a comparatively lower case fatality rate, the unprecedented scale of transmission resulted in a substantial global health and socioeconomic burden.

In the absence of approved vaccines or specific antiviral therapies, and considering the recognized theoretical risk of antibody-dependent enhancement (ADE) observed in preclinical coronavirus vaccine studies, extensive global efforts were initiated to develop safe and effective vaccines using multiple technological platforms, including inactivated virus, recombinant protein, viral vector, DNA, and mRNA approaches. Among these, inactivated virus vaccines emerged as a particularly promising strategy due to their well-established manufacturing processes, preservation of viral antigenicity while eliminating pathogenicity, and feasibility for rapid large-scale production. Accordingly, this study was conducted to evaluate the protective efficacy, safety, and immunogenicity of the inactivated SARS-CoV-2 vaccines (Vero cell) developed by Wuhan/Beijing Institute of Biological Products Co., Ltd in healthy individuals

aged 18 years and above, with the aim of supporting vaccination as a critical long-term measure to control the global spread of COVID-19.

Design:

This is a multicenter, randomized, double-blind, placebo-controlled, Phase 3 trial in 45,000 health participants. The study consisted of 2 periods.

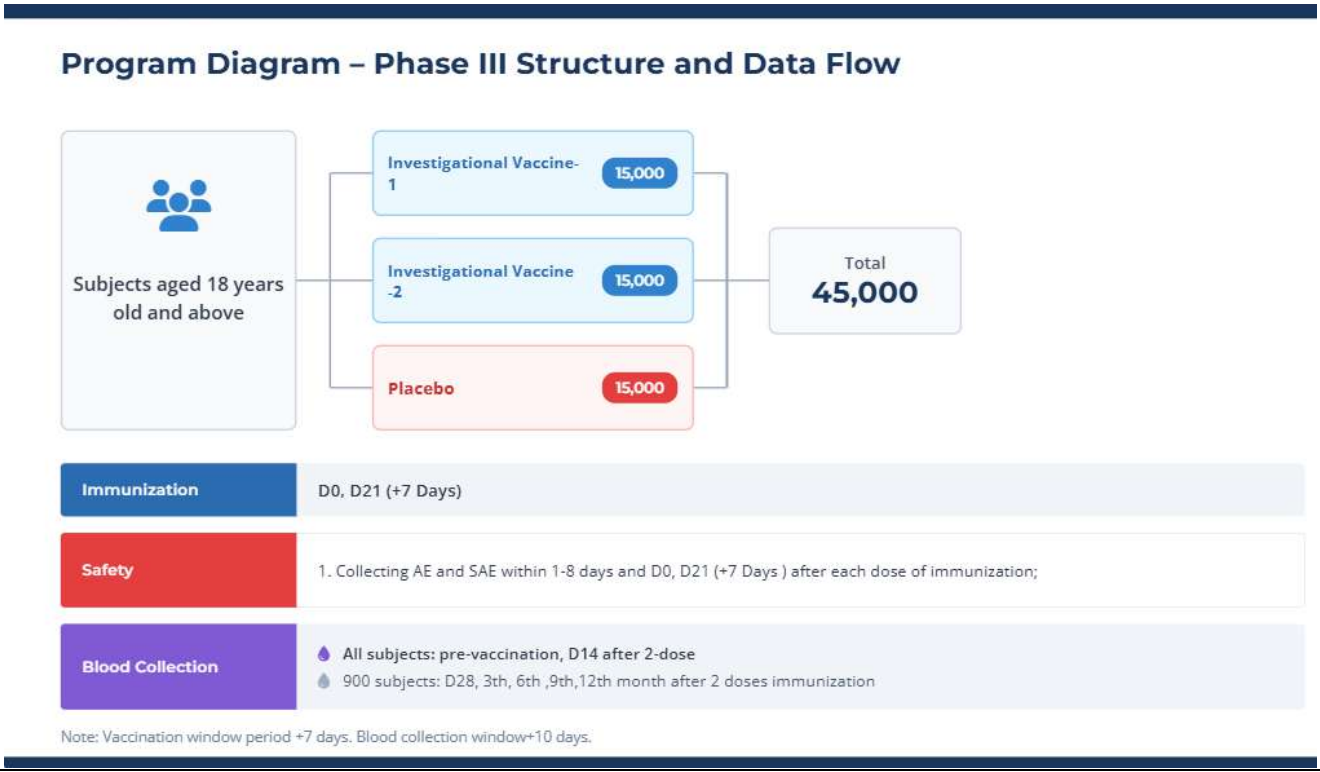
- **A vaccination period of up to 6 weeks**

After voluntary signed consent the subject will be assessed for general health condition and if eligible is confirmed immunization of 2-doses of investigational vaccine or placebo are inoculated to the deltoid muscle of the upper arm according to the D0, D21 (+7 Days) immunization schedule.

- **A safety follow-up period of up to 12 months after vaccination.**

After completion of both vaccination and immediate safety assessment (till visit 10), a period of safety follow-up begins with regular subject visits and phone contacts.

Subjects will be randomized in a 1:1:1 ratio to receive either SARS-CoV-2 WIV04 vaccine or SARS-CoV-2 HB02 vaccine or placebo two times for the period of maximum 28 days.



- ***Recommendation &/ or*** **Questions & Answers: NA**

- **Abbreviation:**

- **ADE:** Antibody-dependent enhancement
- **BP:** Blood pressure
- **BIBP:** Beijing Institute of Biological Products Co., Ltd.
- **CNBG:** China National Biotec Group Company Limited
- **D:** Day
- **g:** Gram
- **GLP:** Good Laboratory Practice
- **GMTs:** Geometric mean titers
- **IB:** Investigator Brochure
- **IFN-γ:** Interferon-gamma
- **IgG:** Immunoglobulin G
- **IL-2:** Interleukin-2
- **IL-6:** Interleukin-6
- **IM:** intraperitoneal
- **IP:** Intraperitoneal
- **Kg:** Kilogram
- **M-dose:** Medium dose
- **MTD:** Maximum tolerated dose
- **ml:** milliliters
- **NA:** Not applicable
- **NtAb:** Neutralizing antibodies
- **PI:** Principle Investigator
- **PRNT:** Plaque reduction neutralization test
- **SARS-COV-2:** Severe acute respiratory syndrome coronavirus 2
- **SC:** Subcutaneous
- **SD rats:** Sprague–Dawley
- **SPF:** Specific Pathogen-Free
- **TNF-α:** Tumor necrosis factor-alpha
- **WIBP**: Wuhan Institute of Biological Products Co., Ltd.
- **WU:** Working Unit (A defined biological potency equivalent to the reference standard)
- **µg:** Microgram

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