Recombinant Ebola Virus Vaccine

(Ad5-EBOV)

Phase II Clinical Trial Protocol

Sponsors： **Beijing Institute of Biotechnology, China**

Tianjin CanSino Biotechnology Inc., China

Version: 1.3 Protocol Date October 5, 2015

TABLE OF CONTENTS

DOCUMENT HISTORY

Version No.	Version Date
1.0	28 July, 2015
1.1	20 August, 2015
1.2	24 September, 2015
1.3	5 October, 2015

Amendment:

Information of the 1st Amendment (20 August, 2015)		
Contents in Original Version (1.0)	**Contents in Revised Version (1.1)**	
Original Contents	**Revised Contents**	**Reason**
None	Pan African Clinical Trials Registry: PACTR201509001259869	The protocol registered
Participants will stay for 30 minutes safety observation after vaccination	Participants will stay for 60 minutes safety observation after vaccination	For safety consideration and the requirement from Pharmacy Board of Sierra Leone
Immunogenicity will be tested on the day 0, 14, and 28 after vaccination	Immunogenicity (ELISA antigen-specific assays and Neutralizing antibody titers against human Ad5) will be tested on the day 0, 14, 28 and 168 after vaccination	Add an immunogenic tests on day 168 to evaluate the antibody persistence
HIV will be examined on day 0 before the vaccination and day 28, 168 after the vaccination	HIV will be examined on day 0 before the vaccination and day168 after the vaccination	The HIV test on day 28 is not necessary since HIV test will be performed on day 0
No DSMB	DSMB will review the reported safety data in the participants after vaccination. During the study period, if an increase of risk for participants is noticed, the DSMB should promptly inform the principal investigator and sponsors. Sponsors, investigators and DSMB will have a panel meeting, and then DSMB will make final decision to pause or call an early termination of the study.	For safety consideration and the requirement from Pharmacy Board of Sierra Leone
HIV infection incidence was not in the criteria for pausing or early termination	Put HIV infection incidence during the study into the Criteria for pausing or early termination	For safety consideration and the requirement from Pharmacy Board of Sierra Leone

Occurrence of grade 3 adverse events associated with vaccination in 15% of participants or more	Remove the item of "Occurrence of grade 3 adverse events associated with vaccination" out of the Criteria for pausing or early termination	DSMB will not review the grade 3 adverse events
The negative result in the urine pregnancy test was not in the inclusion criteria	Add "Non-pregnant females with a negative result in the urine pregnancy test on day of enrollment" in the inclusion criteria	The urine pregnancy test need to be performed on day of enrollment and only those with a negative result could be included in this study
"Family history of seizure, epilepsy" is in the exclusive criteria	Remove the item "Family history of seizure, epilepsy" out of the exclusive criteria	Vaccination would not cause seizures or epilepsy, and have no detrimental effect to an already existing seizures or epilepsy
In the exclusive criteria: "Woman who is pregnant, breast-feeding or positive in β-HCG (human chorionic gonadotropin) pregnancy test (urine) on day of enrollment woman who has been vaccinated and becomes pregnant during the clinical trial will not be included in the data analysis, but will be followed up till delivery to know the outcome of the neonate"	Change to "Woman who is pregnant or breast-feeding"	How to handle the pregnancy during the safety following period of the study has been moved to the Safety follow-up chart, 9.4.6
In the exclusive criteria: "- Hereditary angioneurotic edema or acquired angioneurotic edema" "- Urticaria in last one year" "- Current anti-tuberculosis prophylaxis or therapy"	Remove these three items from the exclusive criteria	Because these three already been covered by the item 4, 7, and 8 in the current exclusive criteria
In the exclusion criteria: "- Prior administration of blood products in last 4 months" "- Prior administration of attenuated vaccine in last 1 month" "- Prior administration of inactivated vaccine in last 14 days"	Remove the item from the exclusive criteria	Vaccination is less likely to be interfered
"ELISpot, HLA, mRNA, MedDRA, PBMC" in the ABBREVIATIONS	Remove these words from the ABBREVIATIONS	These abbreviations are not used in this protocol
The volume of blood collection was not specified	Each visit, participants will be asked to donate 5 ml blood sample. A total of 20 ml blood will be donated for each participants. (Table 7-3-2)	Ensure the volume of serum needed in the immunogenic tests and minimize the risk of participants

Return of diary card on day 28	Return of diary card at day 8 by the safety follow-up staffs	Collect the adverse reactions within the first 7 days after vaccination
the sample size determination in the section 7.4 Sample size	Move the “sample size determination” to the section 9.7.1	Avoiding repetition
Section 8.4 “requirement from participants”	Change to “request from participants”	Clerical error
Section 9.1 “Physical examination, including general physical examination”	Change to “Physical examination, including HIV test and urine pregnancy test”	Clarifying
Section 9.2.1 Randomization	Add more detailed information on the method of randomization in 9.2.1 section Randomization and blinding method	Clarifying
Section 9.2.3 “notify the sponsors at the 24 hours emergency call number (Wei Chen 008613910789661) when the treatment code is broken”	Change to “notify the sponsors at the 24 hours emergency call number (Lihua Hou +23276551560)”	Using an oversea telephone number as emergency call is not suitable
Section 9.2.4 “Serious adverse event and HIV infection screening from28 days to 6 months will be open.”	Change to “Serious adverse event after day 28 and final visit at month 6 will be opened. But the staffs responsible for the laboratory tests will be kept in blinded during the whole study, including the serum tests at month 6.” in section 9.2.3	All the laboratory detection need to be performed in blind.
Section 9.4.1 “Doctors will monitor the vital signs of the participants and teach them to record any adverse reactions or events on the diary card… and record axillary temperature and any adverse events (AEs) for seven consecutive days by their own in a diary card provided by the investigators.”	Revised as “Doctors will monitor the general safety condition of the participants and teach them to observe any adverse reactions or events on the diary card… and report auxiliary temperature and any adverse events (AEs) for seven consecutive days. Each participant will get a digital thermometer, a measure ruler and a cell phone before he or she leave the site for reaction measurement and report.”	Because some participants may be illiterate and could not complete the Diary Card by themselves

5Section 9.4.1 During the first 7 days after vaccination, several trained staffs will collect the safety data from participants daily	Revised as “During the first 7 days after vaccination, several trained staffs will collect the safety data from participants daily by phone or home visit. These trained staffs will record any adverse event reported by participants on the Diary Card. If the symptom of reaction is severe and needs medical intervention, the trained staffs must instruct the participants to seek the treatment in community clinics, and record the treatment on the Diary Card.”	Describe detailed methods of the safety follow-up and the Diary Card recording.
Section 9.4.2.1 “Severe adverse reaction (SAE) is occurrence of any untoward medical during the whole study period that: - Results in death. - Is life-threatening (an event in which the participant is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe).”	Add following three items in the SAE definition: - Results in persistent or significant disability/incapacity. - Requires hospitalization or prolongation of an existing hospitalization. - Is a congenital anomaly/birth defect.”	Give a complete definition of the SAE.
Section 9.4.2.3 “Guidance of Grading Standards in Adverse Reactions Prevention in The Clinical Trial is referred to the FDA guideline.”	Revised as “All adverse events will be graded according to “The standard guidelines for adverse reactions grading of vaccine clinical trials” issued by China state Food and Drug Administration (SFDA).”	Clarifying
Section 9.4.5 “In addition to the initial 24-hour report, a completed, separate SAE report is to be sent to Ministry of Health & Sanitation, Sierra Leone within 48 hours of the event.”	Revised as “The SAE report form must be filled and submited to Pharmacy Board of Sierra Leone and the Sierra Leone Ethics and Scientific Review Committee within 48 hours of the first knowing about the occurrence of the event.”	The SAE report is required by Pharmacy Board of Sierra Leone
Section 9.4.5 not specify that how to handle the death report.	“How will death be handled in terms of verbal and confirmed autopsy? What are the study procedures for verbal and confirmed autopsy?” is added in.	Required by Pharmacy Board of Sierra Leone
Not specify how to report the new HIV infection or pregnancy after vaccination during the follow-up.	Add a section 9.4.6 “Reporting of the occurrence of new HIV infection or pregnancy” to specify the methods for reporting the new HIV infection or pregnancy after vaccination during the follow-up	For the safety concern it is need to be reported

Section 9.7.4 Statistical methods	Add more detailed information about the statistical methods used for safety analysis and immunogenicity analysis in section 9.7.5	Clarifying
Not specify any statistical considerations for the DSMB	Add a section 9.7.6 “Statistical Considerations for the DSMB”	Specify the work of DSMB
Section 10.5 Ownership and publication “All data /information generated in the research center (except the medical records of the participants) belong to sponsors”	Revised as “All data /information generated in the research center (except the medical records of the participants) belong to sponsors and Ministry of Health and Sanitation, Sierra Leone.”	Required by Pharmacy Board of Sierra Leone
Section 10.6 Confidential “representatives of full authorized management such as China FDA have the right to access the clinical trial data”	Revised as “representatives of full authorized management such as Pharmacy Board of Sierra Leone, China FDA have the right to access the clinical trial data”	Required by Pharmacy Board of Sierra Leone
13 APPENDIX “four laboratory standard operation procedure” 14 ANNEX “Informed Consent”	Add more relevant documents to the 13 APPENDIX (Appendix 1-12) Combine the 14 ANNEX with 13 APPENDIX	Add the standard operation procedure lists for both laboratory and site visit, and all the relevant documents

Information of the 2nd Amendment (24 September 2015)		
Contents in Original Version (1.1)	**Contents in Revised Version (1.2)**	
Original Contents	**Revised Contents**	**Reason**
No criteria about the previous vaccination expect for the Ebola vaccine included	Add two items in the exclusion criteria: “- Prior administration of attenuated vaccine in last 1 month” “- Prior administration of inactivated vaccine in last 14 days”	Required by Pharmacy Board of Sierra Leone
Informed consent, collecting demographic information, screening, randomization and administration of investigational vaccine on a same day	Add visit 0 for informed consent and collecting demographic information one day before the other activities of the trial.	Required by Pharmacy Board of Sierra Leone.
Section 9.4.5, no verbal autopsy requirement is described	Add “verbal autopsy must be carried out as per WHO Gudelines using “The 2014 WHO verbal autopsy instrument” (Appendix 12). A copy of autopsy report should be attached with the SAE form.”	Required by Pharmacy Board of Sierra Leone

Subsection 9.4.6, "The participants who become pregnant after the vaccination need to be followed until the baby is born, and then follow the baby for 28 days after birth".	Any pregnancy after vaccination during the study period will be monitored/followed up till delivery and 28 days after delivery of a normal baby. If delivery baby with abnormality, the cause of the abnormality will be investigated by the DSMB.	Required by Pharmacy Board of Sierra Leone

Information of the 3rd Amendment (5 October, 2015)		
Contents in Original Version (1.2)	**Contents in Revised Version (1.3)**	
Original Contents	**Revised Contents**	**Reason**
Subsection 9.4.6, "Any pregnancy after vaccination during the study period will be monitored/followed up till delivery and 28 days after delivery of a normal baby."	Any pregnancy after vaccination during the study period will be monitored/followed up till delivery and 5 years after delivery of a normal baby.	Required by Pharmacy Board of Sierra Leone
No toxicity grading panel for the haematology and clinical chemistry tests	APPENDIX 13 Toxicity grading panel for the haematology and clinical chemistry tests	Required by Pharmacy Board of Sierra Leone
Subsection 7.3 "Each visit, participants will be asked to donate 5 ml blood sample. A total of 20 ml blood will be donated for each participants"	Participants will be asked to donate 8ml blood (uncoagulation: 3ml, coagulation: 5ml) at the first visit, and 5 ml blood sample at the later visits. A total of 23 ml blood will be donated for each participant.	Added haematology and clinical chemistry tests

Brief Title:	A Phase II Clinical Trial to Evaluate the Recombinant Human Type5 Adenovirus Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV)	
Official Title:	A Single-Center, Randomized, Blind, Phase II Clinical Trial to Evaluate the Safety and Immunogenicity of the Adenovirus Type 5 Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV) in Healthy Adults Aged Between 18 and 50 years in Sierra Leone	
Protocol Number:	JSVCT024	
Pan African Clinical Trials Registry:	PACTR201509001259869	
Protocol Date:	October 5, 2015	
Version:	Version 1.3	
Principal Investigator（PI）	Dr. Alie H Wurie	National Ebola Response，Emergency Operations Centre, Ministry of Health & Sanitation, Wilkinson Road, Freetown, Sierra Leone
Co-PI	Dr. Feng-Cai Zhu	Jiangsu Provincial Center For Disease Control and Prevention, China
Clinical Director	Dr James B W Russell	Connaught Hospital, Freetown, Ministry of Health & Sanitation, Sierra Leone

PROTOCOL SUMMARY

Brief Title	A Phase II Clinical Trial to Evaluate the Recombinant Human Type 5 Adenovirus Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV) in Healthy Adults
Official Title	A Single-Center, Randomized, Blind, Phase II Clinical Trial to Evaluate the Safety and Immunogenicity of the Adenovirus Type 5 Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV) in Healthy Adults Aged Between 18 and 50 years in Sierra Leone
Objectives	Preliminarily evaluate the safety, immunogenicity of the Adenovirus Type 5 Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV) after one injection under dosage of 8×10^{10}vp or 1.6×10^{11}vp; to determine appropriate dosage which could support large-scale phase III clinical trials
Target disease	To prevent Ebola virus disease caused by Zaire type Ebola virus infection
Target population	Healthy adults age between 18 and 50 years in Sierra Leone
Sample size	500 participants
Rational and background	Ebola viruses (EBOVs) are known to cause fatal hemorrhagic fever in humans and non-human primates with a mortality rate up to 90%. EBOV is transmitted among humans through close contact with the infected blood, bodily fluids or tissues; also, the intentional release of EBOVs would probably result in mucosal infection by small-particle aerosol dispersion. Five different species of EBOV have been identified: Zaire, Sudan, Ivory Coast, Reston and the newly discovered Bundibugyo. Among these species, infection with Zaire is the most serious and has caused the greatest number of deaths. The EBOV envelope glycoprotein (EBOV-GP) forms spikes on the surface of mature virions, and has been shown to be an effective target for vaccine design. Preclinical studies indicated that both humoral and cellular responses to EBOV

	are very important for viral control and clearance. The Ad5-EBOV is a recombinant replication defective human recombinant Ad5 vector based vaccine expressing Ebola virus glycoprotein (GP) (Guinea, 2014). It is manufactured through virus amplification, purification and formulation with stabilizer and lyophilization. The final product is lyophilized white powder. Immune response would be stimulated after vaccination to prevent the Ebola virus disease.
Previous Clinical Trial Research	A phase I clinical trial which evaluated the safety, tolerability and immunogenicity of the Recombinant Human Type 5 Adenovirus Vector Based Ebola Vaccine was established with a total of 120 participants in Taizhou, Jiangsu Province, started from Dec 28, 2014（ClinicalTrials.gov, number NCT02326194）. This trial evaluated the safety from 0 to 28 days after injection of the low dose 4×10^{10}vp and high dose 1.6×10^{11}vp Ad5-EBOV. It was suggested that dose response relationship of overall adverse drug reactions (mainly local adverse reactions) was apparent. The rate of adverse reactions was higher in high dose groups, however, only local inoculation site and the surrounding pain (most were mild) was observed, meanwhile no adverse reaction greater than Grade 3 was examined. The incidence of adverse reactions was quite similar with the reports of other Ebola vaccines, and good tolerance of the participants of the Ad5-EBOV was found. Ebola glycoprotein-specific antibody titers were significantly increased in participants in the low-dose and high-dose vaccine groups at both day 14 (geometric mean titre 421.4 and 820.5, respectively) and day 28 (682.7 and 1305.7, respectively). T-cell responses peaked at day 14. The result of this clinical trial was published in *Lancet* 2015; 385: 2272–79. Another single-center, open-label phase I clinical trial to evaluate the safety, tolerability and immunogenicity of the recombinant human type 5 adenovirus vector based Ebola vaccine was established with a total of 61 participants of healthy African people in China age between 18 and 60 years in The First

	Affiliated Hospital of Medical College of Zhejiang University, started from Apr 3, 2015 (ClinicalTrials.gov, number NCT 02401373). Two dose groups were set, and the number of participants in the lower dose group (8×10^{10}vp) and the higher dose group (1.6×10^{11} vp) was 31 and 30, total 61 participants in two groups. There was no serious adverse reaction in all the participants. Ebola glycoprotein-specific antibody titers were significantly increased in participants in the low-dose and high-dose vaccine groups at both day 14 (geometric mean titer 1370.9 and 1185.5, respectively) and day 28 (1918.7 and 1684.7, respectively). T-cell responses also peaked at day 14.
Investigational vaccines	Recombinant human type 5 adenovirus vector based Ebola vaccine was jointly developed by Beijing Institute of Biotechnology and Tianjin CanSino Biotechnology Inc. Experimental Vaccine: lyophilized white powder, with recombinant replication defective human recombinant Ad5 vector based vaccine expressing Ebola virus glycoprotein 4×10^{10}vp/ vial. The quality was conformed to the requirements of manufacturing and verification of the recombinant Ebola virus disease vaccine formulated by the sponsors and tested and certified by National Institute for Food and Drug Control (NIFDC), Chinese Food and Drug Administration. Placebo Control: lyophilized white powder, other components were consistent with the experimental vaccine excluded recombinant replication defective human recombinant Ad5 vector based vaccine expressing Ebola virus glycoprotein, tested and certified by National Institute for Food and Drug Control (NIFDC). Immunization schedule: Experimental vaccine group A: one-time inoculation; 1 ml sterilization injection water per dose to dilute 2 vials (4×10^{10}vp/vial), total dose of 1.6×10^{11}vp, one shot in each arm. Experimental vaccine group B: one-time inoculation; 1 ml sterilization injection water per dose to dilute 1 vial (4×10^{10}vp/vial), total dose of 8×10^{10}vp, one shot

	in each arm. Placebo group: one-time inoculation; 1 ml sterilization injection water per dose to dilute 1 vial (0 vp/vial), total dose of 0 vp, one shot in each arm. Inoculation site and inoculation route: The inoculation site was the central lateral deltoid in the upper arm, and inoculation route was intramuscular injection Storage and transport conditions: To store and transport in 2-8 ℃, avoid light.
Trial design	Experimental design: This is a single center, randomized, blind, placebo controlled, phase II clinical trial. Clinical Trial Site: Sierra Leone-China Friendship Hospital in Freetown, Sierra Leone Sample size calculation: According to the results of Phase I clinical trial in Jiangsu, we assume that the positive rate of low-dose and high-dose vaccine groups was 95% and 99.9% respectively and placebo only 5%, the ratio will be set as (high-dose: low-dose: placebo)=2:1:1 in order to assure 80% of certainty when the α value is 0.05. Over 207 people in high-dose vaccine group are needed and 104 people in low-dose and in placebo group respectively. A 15% visit loss rate was assumed so we set the sample size as 250 people in high-dose vaccine group, 125 people in low-dose and placebo group, respectively. The total sample size is 500 people. Randomization and blind operation: Randomization list will be generated by an independent statistician using SAS version 9.1 software or a higher version. In this study, all the participants will be recruited and randomly assigned to receive the Ad5-EBOV as (high-dose: low-dose: placebo) =2:1:1.Blinding will be maintained for all participants, investigators and study staff participating in this study. Research plan:

	A total of 500 participants according to the inclusion criteria will be randomly assigned in 2:1:1 ratio into the experimental vaccine group A (1.6×10^{11}vp), the experimental vaccine group B (8×10^{10}vp) and the placebo group. Participants should stay and be observed for at least 60 minutes after vaccination. They should be closely monitored in the 7 days after vaccination, and urged to complete the safety observation. Immunogenicity will be tested on day 0, 14, 28 and 168 after vaccination so the participants should go back to the hospital in those days. The antibody of HIV will be examined on day 0 and day168 after vaccination. The whole follow-up period for each participant will be 6 months. The occurrence of serious adverse event could be recorded by combination of automatic report from participants and investigator's regular follow-up. Study duration: The whole follow-up period for each participant will be 6 months but some of them may terminate early.
Endpoints	Primary Endpoints: Safety: − Occurrence of solicited adverse reactions within 7 days after vaccination. Immunogenicity: − ELISA antigen-specific assays for antibody responses on day 0, 14, 28 and 168 (month 6). Secondary Endpoints: Safety: − Occurrence of unsolicited adverse reactions within 28 days after vaccination. − Occurrence of serious adverse reaction during the whole follow-up period (6 months). − Infection rate of HIV during the whole follow-up period (6 months). Immunogenicity: − Neutralizing antibody titers response to human Ad5 on day 0, 14, 28, and 168 (6 months).

Scheduled site visits	Visit 0 (day -1): informed consent, and demographic information collecting Visit 1 (day0): screening, physical examination, blood sample taking for HIV test, haematology and clinical chemistry test, and baseline antibody detection; pregnancy test. followed by immunization/administration of vaccine Visit 2 (day 14, ±2 days): assessment of safety, blood sample taking for post-vaccination antibody detection. Visit 3 (day 28, ±3 days): assessment of safety, blood sample taking for post-vaccination antibody detection. Visit 4 (day 168, ±10 days): assessment of safety, blood sample taking for HIV test and post-vaccination antibody detection.
Criteria for pausing or early termination	DSMB will review the reported safety data in the participants after vaccination. During the study period, if an increase of risk for participants is noticed, the DSMB should promptly inform the principal investigator and sponsors. Sponsors, investigators and DSMB will have a panel meeting, and then DSMB will make final decision to pause or call an early termination of the study. Administration of study injections and new enrollments will be paused, if: - One serious adverse event may be associated with vaccination, or - Incidence of new HIV infection post-vaccination reported in one (1) participant during 6 months after vaccination. The study may come to an early termination, if: - One fatal serious adverse event definitely associated to vaccination, or - Incidence of new HIV infection reported in 1% (5 participants) during the 6 months post-vaccination, or - Required by sponsor, or - Required by regulatory authority, or - Required by institutional review board (IRB).
Inclusion Criteria	- Aged between 18 and 50 years - Able to understand the content of informed consent and signed the informed consent

	- Able and willing to complete all the secluded study process during the whole study follow-up period (about 6 months). - Negative in HIV diagnostic blood test on the day of enrollment - Axillary temperature ≤37.0° C on the day of enrollment - Non-pregnant females with a negative result in the urine pregnancy test on day of enrollment - General good health as established by medical history and physical examination.
Exclusion Criteria	- Infected by Ebola virus - Vaccination with other Ebola vaccine - HIV infection or other serious immunodeficiency disease - Allergic history of any vaccination or drugs, or allergic to any ingredient of the Ad5-EBOV, such as mannitol - Family history of brain or mental disease - Woman who is pregnant or breast-feeding - Any acute fever disease or infections in last 7 days - Major congenital defects or not well-controlled chronic illness - Asplenia or functional asplenia - Platelet disorder or other bleeding disorder - Faint at the sight of blood or needles. - Prior administration of immunodepressant or corticosteroids, antianaphylaxis treatment, cytotoxic treatment in last 6 months - Prior administration of other research medicines in last 1 month - Prior administration of attenuated vaccine(s) in the last one month - Prior administration of inactivated vaccine(s) in the last 14 days - Any condition that in the opinion of the investigators may interfere with the participants' compliance or evaluation of study objectives

Role of the sponsor	Sponsors participate in the trial design and the protocol writing, but will not participate in other process of the trial, including data collection, statistical analysis, data interpretation and writing study report.
Sponsor	Person in charge: Wei Chen Beijing Institute of Biotechnology No. 20 Dongdajie Street, Fengtai District, Beijing, People's Republic of China Zip code:100071 Tel:+8613910789661 Fax:+8610-63818253 E-mail: cw0226@foxmail.com
Principal Investigator in Sierra Leone	Dr. Alie H Wurie Senior Specialist & Chairman of Case Management, Address: National Ebola Response, Emergency Operations Centre, Ministry of Health & Sanitation, Wilkinson Road, Freetown, Sierra Leone Tel: +232-76671100 E-mail: wuriealieh@yahoo.com
Co-Principal Investigator in China	Feng-Cai Zhu Jiangsu Provincial Center for Diseases Control and Prevention No. 172, Jiangsu Road, Nanjing, Jiangsu Province, People's Republic of China Zip code: 210009 Tel: +8613951994867 Fax:+8625-83759409 E-mail:jszfc@vip.sina.com
Clinical Director	Dr James B W Russell, Specialist, Connaught Hospital, Ministry of Health & Sanitation, Freetown, Sierra Leone Tel: +232 76 412 442

	E-mail: jamesbwrussell@gmail.com
Clinical Laboratory	Dr. Wenbo Xu Company: Sierra Leone-China Friendship Biological Safety laboratory Address: Sierra Leone-China Friendship Hospital, Freetown Tel:+232-77640363 Email：wenbo_xu1@aliyun.com
Contract Research Organization	Yuan Yong Shenzhen Yingheyuan Medical Technology Development Co., Ltd. 6B-01, Tian-xia Industrial Park, Ma Jia Long, Yi-yuan Road, Nan-shan District , Shenzhen, People's Republic of China Zip code:518052 Tel: +86755-61373860 Fax: +86755-61373008 E-mail: 13382030658@189.cn
Statistical Party	Jie Lin Choice Pharma Medical Information Consultancy (Shanghai) Co., Ltd., Rm. 1202, Tower D Ocean International Center, 60 Dong Si Huan Zhong Road, Chaoyang District, Beijing, People's Republic of China Zip code: 100025 Cellphone: 186 1188 7583 Fax: +86-10-5908-1007 E-mail: jie_lin_2000@yahoo.com

ABBREVIATIONS

AE	Adverse Event
Ad5	Replication Defective Human Adenovirus Serotype 5
AR	Adverse Reaction
ATP	According to Protocol
CFDA	China Food And Drug Administration
CI	Confidence Interval
CRF	Case Report Form
ELISA	Enzyme Linked Immunosorbent Assay
FAS	Full Analysis Set
ITT	Intention To Treat
IEC	Independent Ethics Committee
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
GMFI	Geometric Mean Fold Increase
GP	Glycoprotein
SAE	Serious Adverse Event
SOP	Standard Operation Procedure
Vp	Viral Particle
SS	Safety Set
NIFDC	National Institute for Food and Drug Control

1. OBJECTIVE AND INTRODUCTION

The candidate Adenovirus Type 5 Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV) against Ebola disease is developed by Beijing Institute of Biotechnology and Tianjin CanSino Biotechnology Inc. This is a phase II clinical trial. We are going to evaluate the safety and immunogenicity of the Ad5-EBOV by dose 8×10^{10}vp and 1.6×10^{11}vp in healthy adults aged between 18 and 50 years in Sierra Leone and to determine appropriate dosage which could support large-scale phase III clinical trials.

It was showed that the recombinant virus Ebola vaccine Ad5-EBOV has good immunogenicity in BALB / c mice, guinea pigs and cynomolgus monkeys in preclinical animal tests. Good result was also seen in clinical safety evaluation. The Ad5-EBOV has been approved for clinical trials in China (approval number: 2015L00399). This protocol has been made according to Good Clinical Practice (GCP), the Declaration of Helsinki, and local rules and regulations of China.

At present, according to phase I clinical trials of the vaccine in 120 healthy population at the age of 18-60 years in China, it was showed Ebola vaccine was well tolerated in all participants from 0-28 days. No grade 3 adverse reaction or more was examined. The incidence of adverse reactions was quite similar with reports of other Ebola vaccines. The results showed that high dose vaccine of 1.6×10^{11}vp appeared better immunogenicity compared with the low dose 4×10^{10}vp. Another phase I clinical trial in 61 Africans in Zhejiang Province showed Ad5-EBOV's good safety and immunogenicity. Two dose groups (8×10^{10}vp and 1.6×10^{11}vp) were observed with similar Ebola GP antibody titers.

2. RESEARH FIELD

Study site: Sierra Leone-China Friendship Hospital in Freetown, Sierra Leone

3. RELATED UNITS IN CLINICAL TRIAL

3.1. Sponsor

Beijing Institute of Biotechnology

Tianjin CanSino Biotechnology Inc.

3.2. Investigator

Principal Investigator:

Dr. Alie H Wurie

Senior Specialist & Chairman of Case Management, National Ebola Response

Address: Emergency Operations Centre,

Ministry of Health & Sanitation, Wilkinson Road,

Freetown, Sierra Leone

Phone: +232-76671100; email: wuriealieh@yahoo.com

Co-principal investigator in China:

Dr. Feng-Cai Zhu

Jiangsu Provincial Center for Diseases Control and Prevention

No. 172, Jiangsu Road, Nanjing, Jiangsu Province, People's Republic of China

Zip code: 210009

Tel: 008613951994867

Fax: 008683759409

E-mail:jszfc@vip.sina.com

3.3. Contract Research Organization

Mr. Yuan Yong

Shenzhen Yingheyuan Medical Technology Development Co., Ltd.

6B-01, Tian-xia Industrial Park, Ma Jia Long, Yi-yuan Road, Nan-shan District , Shenzhen, People's Republic of China

Zip code: 518052

Tel: 0086755-61373860

Fax: 0086755-61373008

E-mail: 13382030658@189.cn

3.4. Statistical Party

Dr. Lin Jie

Choice Pharma Medical Information Consultancy (Shanghai) Co., Ltd.,

Rm. 1202, Tower D Ocean International Center,

60 Dong Si Huan Zhong Road, Chaoyang District, Beijing, People's Republic of China

Zip code: 100025

Tel: +86 186 1188 7583

Fax: +86-10-5908-1007

E-mail: jie_lin_2000@yahoo.com

3.5. Clinical laboratory

Dr. Wenbo Xu

Company: Sierra Leone-China Friendship Biological Safety laboratory

Address: Sino-Sierra Leone Friendly Hospital, Freetown

Tel:+232-77640363

Email：wenbo_xu1@aliyun.com

3.6. Clinical Director

Dr James B W Russell,

Specialist, Connaught Hospital, Ministry of Health & Sanitation,

Freetown, Sierra Leone

Tel: +232 76 412 442

E-mail: jamesbwrussell@gmail.com

4. BACKGROUND AND RATIONALE

4.1. Introduction of pathogen

Ebola viruses (EBOVs) are enveloped, non-segmented, negative-strand RNA viruses belonging to the family Filoviridae.[1,2] They are known to cause lethal hemorrhagic fever in humans and non-human

primates with a mortality rate up to 90%.[3,4] Ebola virus disease (EVD), formerly known as Ebola haemorrhagic fever, is a severe, often fatal illness in humans. EBOVs transmit among humans through close contact with the infected blood, bodily fluids or tissues; also, the intentional release of EBOVs would probably result in mucosal infection by small-particle aerosol dispersion.[5]

The current outbreak in west Africa, (first cases notified in March 2014), is the largest and most complex Ebola outbreak since the Ebola virus was first discovered in 1976.[6] There have been more cases and deaths in this outbreak than all others combined. It has also spread between countries starting in Guinea then spreading across land borders to Sierra Leone and Liberia, by air (1 traveler only) to Nigeria, and by land (1 traveler) to Senegal.

The most severely affected countries, Guinea, Sierra Leone and Liberia have very weak health systems, lacking human and infrastructural resources, having only recently emerged from long periods of conflict and instability. On August 8, the WHO Director-General declared this outbreak a Public Health Emergency of International Concern.[7]

Up to now, five different species of EBOV have been identified: Zaire; Sudan; Ivory Coast; Reston; and the newly discovered Bundibugyo.[8] Among these species, infections with Zaire are the most serious and have caused the greatest number of deaths. The virus causing the 2014 West African outbreak belongs to the Zaire species.[3]

4.2. Background of Vaccine

The EBOV envelope glycoprotein (EBOV-GP) forms spikes on the surface of mature virion, and has been shown to be an effective target for vaccine design. GP protein was used as a protective antigen in the Ebola virus vaccine in clinical study process.

Data of our Phase I study on Ad5-EBOV in healthy Chinese people was published on Lancet. 120 participants were enrolled and randomly assigned to receive placebo (n=40), low-dose vaccine (n=40), or high-dose vaccine. Participants were followed up for 28 days. Overall, 82 (68%) participants reported at least one solicited adverse reaction within 7 days of vaccination (n=19 in the placebo group vs n=27 in the low-dose group vs n=36 in the high-dose group; p=0·0002). The most common reaction was mild pain at the injection site, which was reported in eight (20%) participants in the placebo group, 14 (35%) participants in the low-dose group, and 29 (73%) participants in the high-dose vaccine group

(p<0·0001). We recorded no statistical differences in other adverse reactions and laboratory tests across groups. Glycoprotein-specific antibody titers were significantly increased in participants in the low-dose and high-dose vaccine groups at both day 14 (geometric mean titer 421.4 [95% CI 249·7- 711.3] and 820.5 [598.9-1124.0], respectively; p<0·0001) and day 28 (682.7 [424.3-1098.5] and 1305·7 [970.1-1757.2], respectively; p<0·0001). T-cell responses peaked at day 14 at a median of 465·0 spot-forming cells (IQR 180.0-1202.5) in participants in the low-dose group and 765·0 cells (400·0-1460·0) in those in the high-dose group. 21 (18%) participants had mild fever (n=9 in the placebo group, n=6 in the low-dose group, and n=6 in the high-dose group). No serious adverse events were recorded. It was proved the vaccine is safe and has high immunogenicity. GP specific humoral immune was stimulated on day 14 after primary vaccination in high dose group.

Another single-center, open-label phase I clinical trial to evaluate the safety, tolerability and immunogenicity of the recombinant human type 5 adenovirus vector based Ebola vaccine was established with a total of 61 participants of healthy African people in China age between 18 and 60 years in The First Affiliated Hospital of Medical College of Zhejiang University, started from Apr 3, 2015. Two dose groups were set, and the number of participants in the lower dose group (8×10^{10}vp) and the higher dose group (1.6×10^{11} vp) was 31 and 30, total 61 participants in two groups. There was no serious adverse reaction in all the participants. Ebola glycoprotein-specific antibody titers were significantly increased in participants in the low-dose and high-dose vaccine groups at both day 14 (geometric mean titer 1370.9 and 1185.5, respectively) and day 28 (1918.7 and 1684.7, respectively). T-cell responses also peaked at day 14.

4.3. Advantage of Ad5-EBOV

Since its first outbreak occurred in 1976, Zaire Ebola virus has been associated with 14 outbreaks reported up to 2014. The Zaire Ebola virus in 2014 causing the most serious outbreak was considered to be a new epidemic strain, with GP homology of the gene was only 97.6%, compared to the GP gene of the strain in 1976.[11]All the other Ebola Vaccines undergoing the clinical trial were developed based on the Zaire-Mayinga (1976 strain). This experimental Ad5-EBOV is the first Ebola vaccine developed according to the 2014 epidemic strain. Besides, the Ad5-EBOV is lyophilized white products which could be stored at 2-8°C. The lyophilized formulation of the vaccine may be more suitable for use in

some area where the cold chain system is incomplete.

The results of clinical study on chimpanzee adenovirus vector Ebola vaccine published preliminary in the New England Journal of Medicine on Nov 27th, 2014. The dose related immune response was demonstrated. Because the gene sequences of Ebola virus glycoprotein in this vaccine is from Zaire- Mayinga (1976), geometric mean titer of the antibody to Zaire-Mayinga (1976) glycoprotein at 2 and 4 weeks respectively were 376 and 2037. However, geometric mean titer of the antibody to Zaire- Guinea (2014) glycoprotein at 2 and 4 weeks were respectively 177 and 623, indicating that there is a difference between the two antigen immunogenicity, prompted by Zaire-Guinea (2014) envelope glycoprotein in 2014 epidemic may have better protection.

5. PRECLINICAL STUDIES WITH CANDIDATE AD5-EBOV

5.1. Preclinical immunogenicity evaluation

5.1.1. Immunogenicity in mice

5.1.1.1. Dosage

Firstly, the immune dose of Ad5-EBOV was explored. Immune BALB/c mice with Ad5-EBOV 10^6 or 10^7 IFU respectively, and peripheral blood were detected in week 2, 3, 4, 6, 8, 10 by ELISA. The dependence on the immune dose and level of serum antibody was observed. Antibody level is very low in 10^6 IFU immunized mice, while it increased significantly in 10^7 IFU immunized group. So 10^7 IFU was the assured dose after the immunological evaluation.

5.1.1.2. Inoculation times

10^7 IFU Ad5-EBOV were immunized at one time or twice with the time interval 4 weeks. Bleed the mice at specific time points to detected serum antibody levels. It can be seen that the antibody levels of the secondary immunization increased apparently than the primary. Whether it was primary or secondary immunization, the serum antibody levels of the mice during the past six months were still stable. Thus, the primary immunization can achieve enough immune effect.

5.1.1.3. Neutralization antibody

Pseudovirus was used in detection of neutralizing antibodies. The Ebola pseudovirus packaged via the method of co-transfection as follows: the plasmid pDC316-AGPZ expressed Ebola virus full-length envelope glycoprotein and HIV skeleton plasmid pNL4-3.Luc-R-, E- with deletion of vpR and env gene expressed firefly luciferase were transfected into 293T cells using TurboFect transfection reagent. 48 hours later, cell culture medium supernatant was collected and froze in -70℃ refrigerator. The plasmid VSV-G and pNL4-3.Luc-R-, E- were transfected into 293T cells to package vesicular stomatitis virus pseudotyped virus as control. The 293T cells would be infected by pseudovirus by 1:10. After 48 hours, firefly luciferase assay system was applied according to the attached manual operation method for detecting levels of luciferase expression in pseudovirus infected cells. After primary immunization with Ad5-EBOV, blood samples were collected at week 6, and the neutralizing antibody was detected based on the detection of IgG positive. Compared to the negative serum, Zaire Ebola pseudotyped virus can be obviously neutralized by serum of mouse which immunized with recombinant Ad5-EBOV at 1:10 dilution.

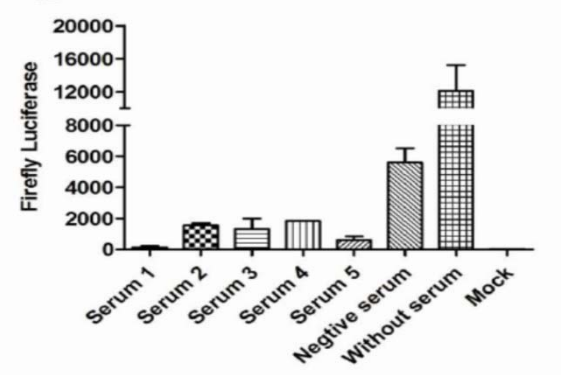


Fig.5.1.1.3 Levels of neutralizing body in Ad5-EBOV immunized mice

5.1.1.4. Cellular immune response

The level of cellular immune response of 10^7 IFU Ad5-EBOV immunized mice was detected on day 28 by flow cytometry assay and ELISPOT. It was showed obviously cellular immune response was found in the immunized group, while the placebo group was not detected as below.

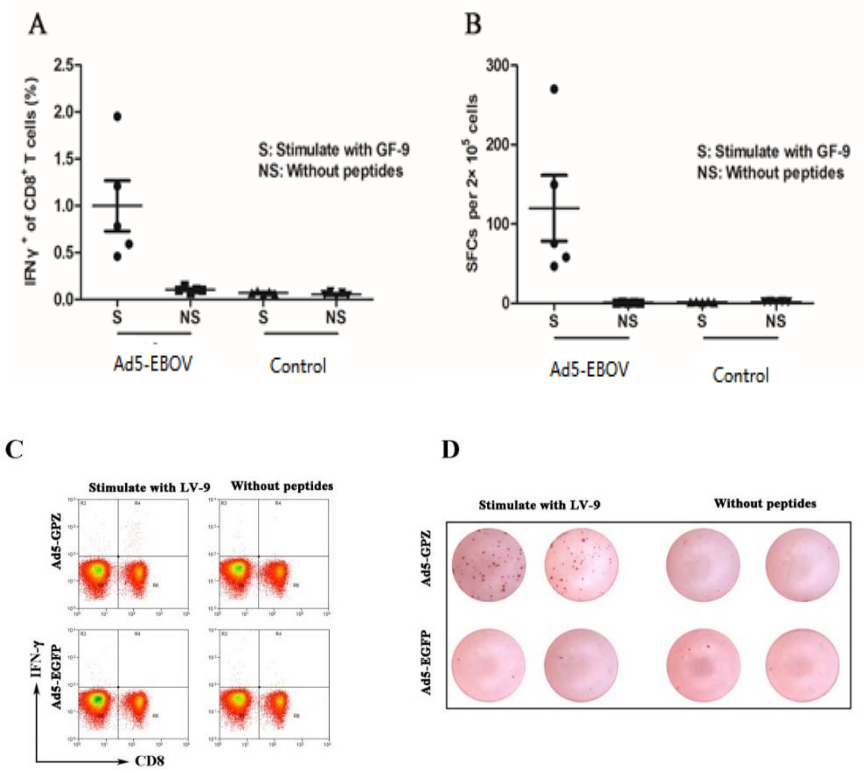


Fig.5.1.1.4 The level of cellular immune response in Ad5-EBOV immunized mice

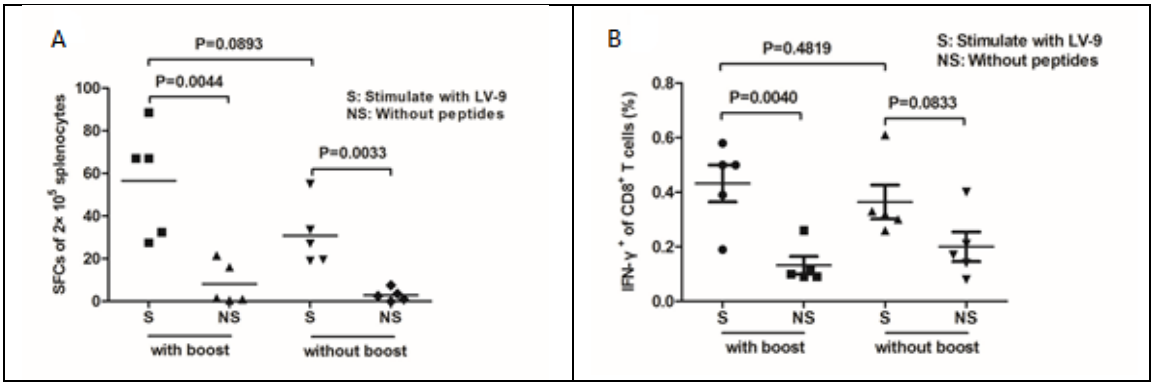
A. Summary of IFN-γ level by flow cytometry assay;

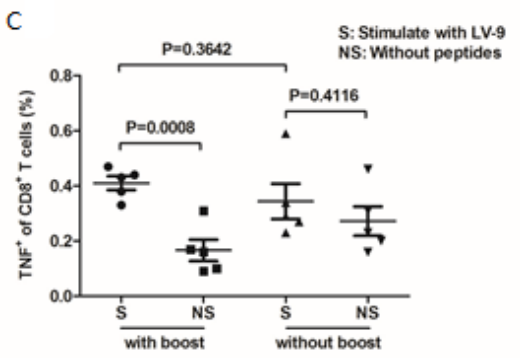
B. Summary of IFN-γ level by ELISPOT;

C. Representative results in flow cytometry assay;

D. Representative results in ELISPOT.

We've checked whether boost or not could affect the cellular immunity in mice which were Ad5-EBOV-immunized nearly half a year. The level of cellular immunity was tested by flow cytometry assay and ELISPOT as below. The cellular immune response in the Ad5-EBOV immunized mice after six months' primary immunization could still remain on a certain level. Obvious increase in boosting immunized mice than the ones only primary immunized was founded. Still obvious difference was showed to non-stimulated cells.





A. IFN-γ positive cells in splenocytes (ELISPOT);

B. IFN-γ positive cells in CD8+ cells(FCA);

C.TNF-α positive cells in CD8+ cells(FCA);

Fig.5.1.1.5 Maintenance of cellular immune level in Ad5-EBOV immunized mice

5.1.2. Immunogenicity in Guinea pigs

36 guinea pigs were grouped for evaluating the immunogenicity of the Ad5-EBOV, including blank group, 10^7IFU group and 10^6IFU group. The IgG titer was assayed 0, 2 and 4 weeks after inoculation and titers in week 4 showed significantly higher than week 2.

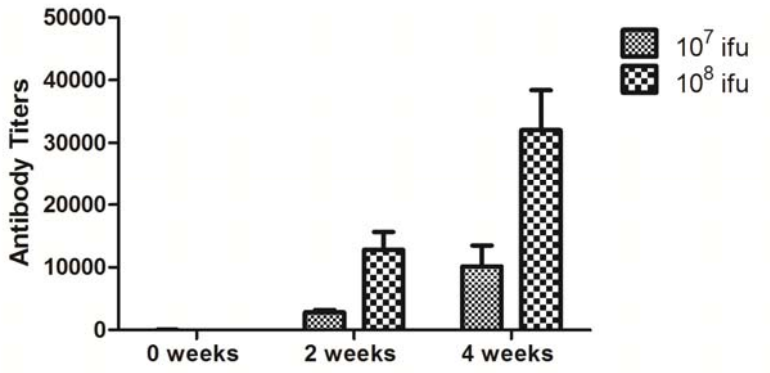


Fig.5.1.2 Level of antibody titers in Ad5-EBOV immunized Guinea pigs

5.1.3. Immunogenicity in cynomolgus monkeys

18 cynomolgus monkeys were grouped for evaluating the immunogenicity of the Ad5-EBOV on 2 week and 4 week after immunization, including blank group, 2×10^{10}vp group and 2×10^{11}vp group. The IgG titers reached high level on 2 week and there was no significant difference between 2×10^{10}vp and 2×10^{11}vp groups. Furthermore there was no significant difference for IgG titer between week 2 and week 4.

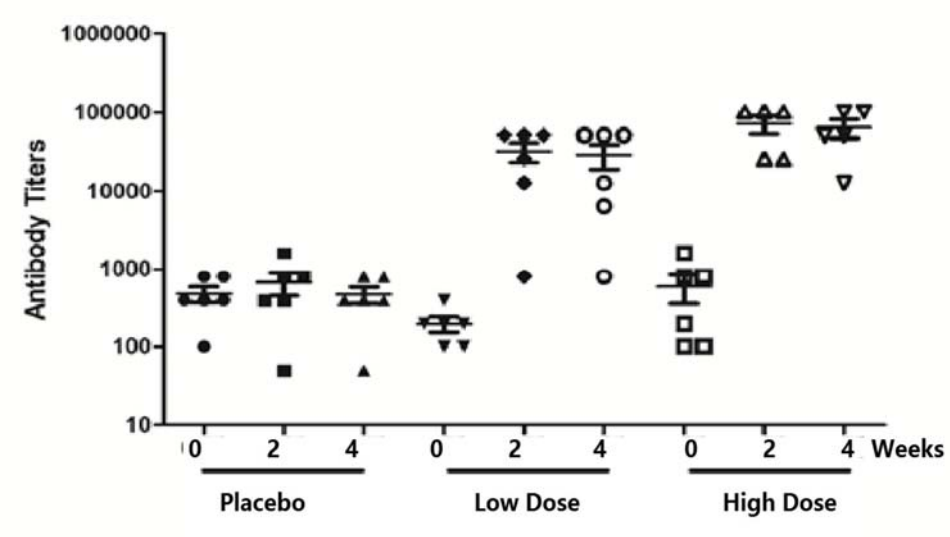


Fig.5.1.3 Level of antibody titers in Ad5-EBOV immunized cynomolgus monkeys

5.1.4. Efficacy in Guinea pigs

Protection of Ad5-EBOV in guinea pigs was processed in the National Microbiological Laboratory of Public Health Agency in Canada. Fifteen guinea pigs (Hartly strains, female, weight in 420-580g) were randomly divided into 3 groups, with Ad5-EBOV intramuscular injection in vaccine group (one of 4×10^{9}vp group and the other 4×10^{10}vp group, 6 in each), control group were injected with Ad5-lacZ (a kind of recombinant human type 5 adenovirus expressed beta galactosidase, 3 in this group). 28 days after immunization, 1ml of 1000× LD50 Zaire Ebola virus (EBOV) was injected intraperitoneal. Survival rate and weight change were observed within 16 days after challenge, and the survival rate was then observed for another 12 days, for a total of 28 days. The result showed guinea pigs in control group all died in 6-8 days after challenged and guinea pigs in the vaccine group of both high and low dose survived and their weight had been growing after EBOV challenged, suggesting efficient protection of Ad5-EBOV vaccine. The survival and weight of guinea pigs were shown in figure 5.1.4.1 and 5.1.4.2

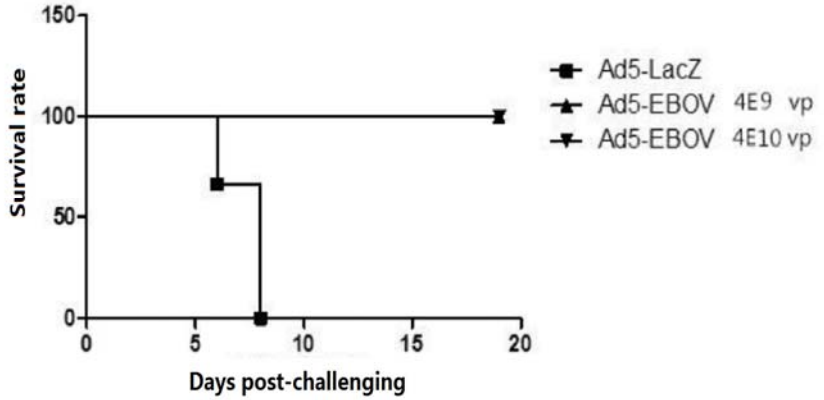


Fig.5.1.4.1 Survival curve of guinea pigs p.i.

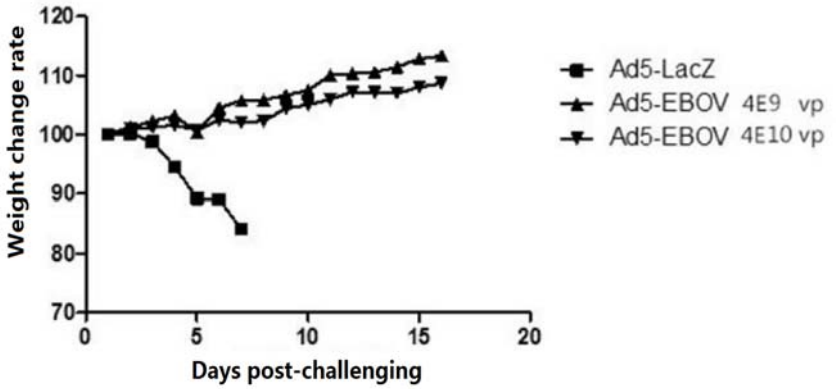


Fig.5.1.4.2 Weight change of guinea pigs p.i.

5.1.5. Efficacy in cynomolgus monkeys

Protection of Ad5-EBOV in nonhuman primates was processed in the National Microbiological Laboratory of Public Health Agency in Canada. 8 cynomolgus monkeys (female, weight in 3-4kg) were randomly divided into 3 groups, with Ad5-EBOV intramuscular injection in vaccine group (one of 4×10^{10}vp group and the other 2×10^{11}vp group, 3 in each), control group were injected with Ad5-TB (a kind of recombinant human type 5 adenovirus expressed TB antigen, 2 in this group). 28 days after immunization, 1000× LD_{50} Zaire Ebola virus (EBOV/Makona/CO7) was injected intraperitoneal \Survival rate and weight change were observed within 16 days after challenge, and the survival rate was then observed for another 12 days, for a total of 28 days. The result showed monkeys in control group all died in 8 days after challenged and monkeys in the vaccine group of both high and low dose survived.

5.2. Safety evaluation in preclinical research

5.2.1. Acute toxicity in mice

The acute effects of the candidate Ad5-EBOV had been examined in Kunming mice. Mice were assigned to a control group or a treatment group, with ten (five female and five male) in each group. Mice in the treatment group were injected intramuscularly a maximum single-dose of Ebola vaccine at 4×10^{12}vp/kg, and the control mice received the same volume of control without virus particles. The acute toxic reactions were observed and recorded for 14 days after dosing. General clinical observations: no animal death was observed in any group after dosing in the 2 weeks, and there were no abnormalities in animal general clinical signs, including behavior, stool appearance and color, skin, mucous membranes, breathing, heartbeat, fur, eyes, nose and limbs. Animals in the treatment group showed stable growth in body weight, and there was no significant difference as compared to the control group. Mice were sacrificed for gross necropsy at the end of the observation period, and no obvious abnormalities in the main organs and tissues were observed in both groups. In summary, in this experimental condition, the maximum tolerated dose of single intramuscular injection of recombinant Ebola virus vaccine was greater than 4×10^{12}vp/kg for the mice (equivalent to 1500 times of human highest clinical dose in body weight).

5.2.2. Repeated intramuscular injection toxicity test in cynomolgus monkeys

The safe dosage of the Ad5-EBOV was examined in the cynomolgus monkey. Monkeys were assigned to three groups: control group, 2×10^{10}vp group and 2×10^{11}vp group, 6 cynomolgus monkeys (three female and three male) of each group. Two doses of the vaccine or placebo control were planned to be given to the animals (intramuscular injection each 4 weeks). After dosing, general clinical observations, body weight, food consumption, body temperature, electrocardiogram, haematology, clinical biochemistry, urine chemistry, immunology parameters and histopathology would be studied. The current results were showed as follows. Animals in each group showed no obvious abnormalities in general clinical observations, body weight, food consumption, body temperature, electrocardiogram, haematology, clinical biochemistry and urine chemistry. Two weeks after dosing, specific serum antibody of the vaccine in low and high groups were both significantly elevated. In conclusion, the current results showed that in this experimental condition, intramuscular injection of the low and high doses of Ad5-EBOV in cynomolgus monkeys did not pose a major risk of toxic effects, and that the

monkeys were well tolerated to the vaccine.

5.3. Summary for preclinical studies

The Ad5-EBOV, developed by Beijing Institute of Biotechnology/Tianjin CanSino Biotechnology Inc., is a replication-defective adenovirus type 5 vaccine which expresses Ebola virus Zaire (Ebola virus/H sapiens-wt/GIN/2014/Makona-C15) envelope glycoprotein. The Ad5-EBOV was a replication-defective virus, which could not replicate in vivo. Previous study showed humoral and cellular responses played important role in protection from infection.[13] Immunological test of the Ad5-EBOV on animals showed good immunogenicity and safety without adverse reaction in animals, which meant the Ad5-EBOV was ready for clinical evaluation.

5.4. Research of phase I

5.4.1. Phase I clinical trial in healthy people in China (Taizhou)

A Phase I clinical trial of Ad5-EBOV vaccine in Chinese healthy people was established by Jiangsu Provincial Center for Disease Control and Prevention in Dec, 2014. This clinical trial was double-blind, dose-escalation, placebo-controlled in order to evaluate the safety, tolerability and immunogenicity of the recombinant human type 5 Adenovirus vector based Ebola vaccine (Ad5-EBOV) in healthy adults aged between 18 and 60 years in China.

According to the sequential principle, research was started from the low dose to the high dose progressively. 60 participants were randomly assigned and inoculated with low dose (4×10^{10}vp) and placebo-controlled, according to 2:1 proportion firstly. 7 days after vaccinated safety and tolerability was assured in low dose and placebo control groups, another 60 participants were randomly assigned and inoculated with high dose (1.6×10^{11}vp) and placebo-controlled according to 2:1 proportion, too. Each participants was immunized once and randomly vaccinated or placebo. Safety evaluation of vaccine depended on the local adverse reaction, occurrence rate of systemic adverse reactions, severity and association between adverse events with the correlation of experimental vaccine. At the same time, vaccine safety was evaluated by detection results of the blood routine, blood biochemical (liver, kidney function) and blood coagulation comprehensively.

Level of anti-Zaire Ebola virus envelope glycoprotein antibody and neutralizing antibody to

recombinant replication defective human type 5 adenovirus were detected on 0, 14, 28 days after vaccinated to evaluate the immunogenicity of different doses. At present, it was shown that overall adverse reaction and local adverse reactions of dose-response relationship were obvious from 0-28 days with higher adverse reaction rate in the higher dose group. Only grade 1 (mild) local responses like the inoculation site and the surrounding pain were observed, there was no vaccination related grade 3 or more adverse reactions, similar with other reports of Ebola vaccine suggesting good tolerance of recombinant Ebola vaccine. Immunogenicity results showed that GP antibody positive rate and GMT levels in both the low and high dose group were 95.00%, 100.00% and 1:682.68, 1:1305.66 on 28 days after immunization, high dose of 1.6×10^{11}vp vaccine showed a good immunogenicity, compared with the low dose 4×10^{10}vp.

5.4.2. Phase I clinical trial in healthy African people in China (Hangzhou)

Another single-center, open-label phase I clinical trial to evaluated the safety, tolerability and immunogenicity of the Recombinant Human Type 5 Adenovirus Vector Based Ebola Vaccine was established with a total of 60 participants content of healthy African people in China aged between 18 and 60 years in The First Affiliated Hospital of Medical College of Zhejiang University, started from Apr 3rd, 2015. Two dose groups were set and the number of participants in the lower one (8×10^{10} vp) and the higher (1.6×10^{11} vp) was 31 and 30 respectively, 61 in total. There was no serious adverse reaction in all the participants. Ebola glycoprotein-specific antibody titers were significantly increased in participants in the low-dose and high-dose vaccine groups at both day 14 (geometric mean titer 1370.9 and 1185.5, respectively) and day 28 (1918.7 and 1684.7, respectively). T-cell responses also peaked at day 14.

5.5. Production technology

The recombinant human type 5 adenovirus vector based Ebola vaccine which expresses Ebola virus Zaire (Ebola virus/H sapiens-wt/GIN/2014/Makona-C15) envelope glycoprotein was lyophilized-dried powder produced by passage, purification, stabilizer addition and freeze drying. Humoral and cellular responses could be caused after vaccination to prevent Ebola virus disease.

The Recombinant Human Type 5 Adenovirus Vector Based Ebola Vaccine which expresses Ebola virus

Zaire (Ebola virus/H sapiens-wt/GIN/2014/Makona-C15) envelope glycoprotein could be copied and enlarged in 293 cell line expressed E1 protein of Adenovirus. Non-serum medium was applied to culture HEK293 SF-3F cells. Seed cells were incubated in the shake flask then transferred to the bioreactor for expansion. When the density and volume of cells came to a certain extent, virus was added to infect cells. 42-46 hours later, cells were collected and froze to release the virus. Nucleic acid enzyme was processed into virus liquid, the virus purified via anion chromatography (Q Sepharose HP), and composite mode chromatography (capto core 700 size exclusion column).Virus was processed by filtration sterilization after added sucrose and mannitol to obtain the original solution.

5.6. Formulation

It was indicated that suitable freeze-drying protection agent could be used to develop the freeze-drying dosage form of the adenovirus vector vaccine. After comparison of main parameters such as appearance, moisture, loss rate of virus infection titer, vaccine safety, efficacy and pH value, we ultimately determined the lyophilized preparation of stabilizer, freeze dry curve and dose of virus, and the final product formulation is 50mg / mannitol, 25-50mg/ml sucrose, 25-50mM saline, 0.01% (V / V) Tween-80, 21mMMgCl, 10 mm PBS.

5.7. Stability research

The Ad5-EBOV vaccine is a freeze-dried powder with diluent for injection. Its storage condition is 2-8℃. It should be transported avoiding light. Preliminary stability study at 37℃ for 2 weeks indicated virus titer, virus particle number, virus purity, moisture, pH value and bacterial endotoxin items were stable and consistent with the quality standards, and no significant difference prior to the initial state of 2 weeks before. The study at 4℃ for 5 months showed that all the indicators were in accordance with the standards. Virus titer decline for 1 week at 37 ℃ should be less than 1.0 log and the period of validity of the vaccine should be lasted for 18 months according to the verification requirements of lyophilized viral vaccine in the 2010 version of "Chinese Pharmacopoeia" (Vol.3). Virus titers of at 37℃ for 2 weeks did not decrease significantly. Above all, we set the preservation condition as tentatively scheduled for 2-8℃ for 1 year and transportation with light preservation should be avoided throughout transportation.

5.8. Quality research and verification

The quality standard of the original solution and vaccine were formulated according to the 2010 Edition "China Pharmacopoeia" (third) and European Pharmacopoeia. The sample batch of clinical trial (201501002) was commissioned and verified by the National Institute for Food and Drug Control (NIFDC), and the results were in accordance with the quality standards. The self-check results of the vaccine are consistent with the results of NIFDC.

Vaccines quality standard inspection

No.	Verified items	Standard	201501002 （NIFDC）
1	Physical Appearance	White or milky white loose body, after adding the diluents with labeled amount, dissolved as a colorless transparent liquid after shaking , no visible foreign particles.	Conforms with the regulations
2	Titration on 293 cells (IFU/dose)	$\geqslant 1\times 10^{8}$	6.7×10^{8}
3	The identification of Viral vectors	Amplified fragment size should be consistent with the theory(735bp)by PCR	Conforms with the regulations
4	pH	7.0～8.0	7.4
5	The identification of target gene	Amplified fragment size should be consistent with the theory(2000bp)by PCR	Conforms with the regulations
6	Moisture (%)	≤3.0	0.7
7	Detection of EBOV Expression from Infected 293 cells	The antigen expression should be positive by Western blot method	Conforms with the regulations
8	Sterility test	Sterility	Conforms with the regulations
9	Virus particle number (vp/dose)	$\geqslant 2.0\times 10^{10}$	4.6×10^{10}
10	Bacterial Endotoxin	≤50	<10

	Detection(EU/dose)		
11	Purity (%)	≥95.0	98
12	Abnormal Toxicity Detection	Animals should be fully healthy within the observation period, and no abnormal reaction, Each animal weight gain maturity.	Conforms with the regulations
13	Osmolarity (mosm/kg)	400～650 mosm/kg	440

5.9. Package

The vaccine will be packed in a box with labels. The label contains at least the following information: vaccine name, lot number and duration of vaccine, and vaccine preservation conditions and “only for clinical studies”. Sample of label on the vial:

Only for clinical studies

Recombinant Human Type 5 Adenovirus Vector

Based Ebola Virus Disease Vaccine

4×10^{10}vp/vial

Lot：201501002

Date：2015.01.28

Exp： 2016.01.28

Storage: at 2～8℃, avoid light

Beijing Institute of Biotechnology

Tianjin CanSino Biotechnology Inc.

Sample of label on the packaging box:

Only for clinical studies

Recombinant Human Type 5 Adenovirus Vector

Based Ebola Virus Disease Vaccine

For one individual

Lot：201501002

Date：2015.01.28 Exp：2016.01.28

Storage: light avoided 2～8℃

- Usage and dosage: add 1ml water for injection solution, use immediately after shaking, intra-muscular injection at the lower edge of deltoid muscle in upper arm of 0.5ml.

Contraindications:(1) Allergic to any ingredient of the Ad5-EBOV (2) Acute disease, severe chronic diseases, chronic disease acute exacerbation and fever (3) Seizure, epilepsy, brain or other mental disease(4)Adults over the age of 18, excluding pregnant women.

Adverse reactions:

Notice:

(1) Cracked vials, unclear or failure label, abnormal appearance after rehydration are not allowed to use; (2) Drug as adrenaline should be equipped for use in case of severe allergic reaction. The participants should be observed at least 60 minutes after injection.

Producers：Beijing Institute of Biotechnology/Tianjin CanSino Biotechnology Inc.

Address：No. 20 Dongdajie Street, Fengtai District / No. 185 South Ave., TEDA West District

5.10. Transportation and Storage

The vaccine must be stored in a safe, locked place to avoid unauthorized access. The vaccine storage conditions must be assessed in study center to ensure that the vaccine is stored under appropriate conditions in the study. The temperature of vaccine transportation from Beijing Institute of Biotechnology/Tianjin CanSino Biotechnology Inc.to the research center, the remaining vaccine after inoculation back to the research center should be kept at 2-8℃. When the vaccines are received, the quantity, quality and maintenance of the cold chain must be checked, and the "vaccine delivery" form should be filled in.

The temperature of the monitoring instrument, transport and storage of the vaccine should be monitored (Am and PM manually) daily. Once the temperature deviation happens, as the temperature over the provisions of the range of 2-8℃, the investigators and sponsors should be immediately informed, and the "cold chain deviation report form" should be filled in, too. The temperature-deviated vaccine should be identified, placed separately and suspended. Continual usage of vaccines must be under written approval by Beijing Institute of Biotechnology/Tianjin CanSino Biotechnology Inc. Vaccine out of requirements should be on-site sequestration.

6. STUDY OBJECTIVES

To evaluate the safety and immunogenicity of the Adenovirus type 5 vector based Ebola virus disease vaccine (Ad5-EBOV) in healthy adults in Sierra Leone, and determine the appropriate immune dose.

7. STUDY DESIGN

7.1. Design methods

This is a single-center, randomized, blind phase II clinical trial. Blind labeled method is performed to keep whole study randomized and blind. The experimental vaccine groups A, B and placebo are randomly encoded by 2:1:1 ratio.

7.2. Study endpoints

7.2.1. Primary endpoints

7.2.1.1. Safety

- Occurrence of solicited adverse reactions within 7 days after vaccination.

7.2.1.2. Immunogenicity

- ELISA antigen-specific assays for antibody to GP responses on day 0, 14, 28 and 168 (month 6).

7.2.2. Secondary endpoints

7.2.2.1. Safety

- Occurrence of unsolicited adverse reactions within 28 days after vaccination.
- Occurrence of serious adverse reaction during the whole follow-up period (6 months).
- Post-vaccination Rate of infected with HIV.

7.2.2.2. Immunogenicity

- Neutralizing antibody titers response to human Ad5 on day 0, 14, 28 and 168 (month 6).

7.3. Study Procedures

From beginning to the end of the study, there are 5 times visits of participants. The visit time, window period and visit content are shown in table 7-3-1 and 7-3-2.

Table.7-3-1 Visit plan for recombinant Ebola virus vaccine (Ad5-EBOV)

Visit	Visit time	Time window
Visit 0 (V0)	One day before the vaccination	—
Visit 1 (V1)	Vaccination at Day 0	—
Visit 2 (V2)	Day 14 after vaccination	±2 days
Visit 3 (V3)	Day 28 after vaccination	±3 days
Visit 4 (V4)	Day 168 (Month 6) after vaccination	±10 days

Table.7-3-2 Visit content and schedule for recombinant Ebola virus vaccine (Ad5-EBOV)

Content		V0	V1	V2	V3	V4
		Day -1	Day 0	Day 14	Day 28	Day 168
1	Collecting demographic information	☆				
2	HIV informed consent	☆				
3	Study Informed consent	☆				
4	Physical examination					

	Height, weight measurement		☆			
	Urine pregnancy tests (female)		☆			
	HIV antibody screening		☆			☆
5	Axillary temperature measurement		☆			
6	Inclusion and exclusion criteria for screening		☆			
7	Allocation of vaccine ID		☆			
8	Record on the Visit Record Form		☆			
9	Blood collection *:					
	Haematology and clinical chemistry tests ^		☆			
	Detection of anti-Zaire Ebola virus envelope glycoprotein antibody		☆	☆	☆	☆
	Detection of neutralizing antibodies to recombinant replication defective type 5 adenovirus		☆	☆	☆	☆
10	Vaccination		☆			
11	Observation for 60 minutes post-vaccination		☆			
12	Instruction on observation of solicited /unsolicited symptoms		☆			
13	Record of solicited / unsolicited symptoms post-vaccination		☆	☆	☆	
14	Report serious adverse events		☆	☆	☆	☆
15	Return of diary card		Return by safety follow-up staffs at day 8			

*Participants will be asked to donate 8ml blood (uncoagulation: 3ml, coagulation: 5ml) at the first visit, and 5 ml blood sample at the later visits. A total of 23 ml blood will be donated for each participant.

^ The toxicity grading panel for the haematology and clinical chemistry tests are provided in Appendix 13.

7.4. Sample size

We set the sample size as 250 people in high-dose vaccine group, 125 people in low-dose and placebo group, respectively. The sample size is 500 in total.

7.5. Criteria for pausing the study or an early termination

DSMB will review the reported safety data in the participants after vaccination. During the study

period, if an increase of risk for participants is noticed, the DSMB should promptly inform the principal investigator and sponsors. Sponsors, investigators and DSMB will have a panel meeting, and then DSMB will make final decision to pause or call an early termination of the study.

Administration of study injections and new enrollments will be paused, if:

- One serious adverse event may be associated with vaccination, or
- Incidence of new HIV infection post-vaccination reported in one (1) participant during 6 months after vaccination.

The study may come to an early termination, if:

- One fatal serious adverse event definitely associated to vaccination, or
- Incidence of new HIV infection reported in 1% (5 participants) during the 6 months post-vaccination, or
- Required by sponsor, or
- Required by regulatory authority, or

Required by institutional review board (IRB).

7.6. Duration of study

The whole follow-up period for each participant will be 6 months.

8. PARTICIPANTS

8.1. Participants selection

Healthy local 18-50 years old residents in Sierra Leone will be informed by ethical review committee approved consent to be a volunteer. After physical examination and screening according to the following inclusion and exclusion criteria, they will be eligible to participate in this study. Any employee who carried out the research, the relevant researchers, and the contract research organization (CRO) could not be the participants. Following inclusion and exclusion criteria will be used to select the eligible participants for this study.

8.2. Inclusion criteria

- Aged between 18 and 50 years

- Able to understand the content of informed consent and signed the informed consent
- Able and willing to complete all the secluded study process during the whole study follow-up period (about 6 months).
- Negative in HIV diagnostic blood test on day of enrollment
- Axillary temperature ≤37.0° C on the day of enrollment
- Non-pregnant females with a negative result in the urine pregnancy test on day of enrollment
- General good health as established by medical history and physical examination.

8.3. Exclusion criteria

- Infected by Ebola virus
- Vaccination with other Ebola vaccine
- HIV infection or other serious immunodeficiency disease
- Allergic history of any vaccination or drugs, or allergic to any ingredient of the Ad5-EBOV, such as mannitol
- Family history of brain or mental disease
- Woman who is pregnant or breast-feeding
- Any acute fever disease or infections in last 7 days
- Major congenital defects or not well-controlled chronic illness
- Asplenia or functional asplenia
- Platelet disorder or other bleeding disorder
- Faint at the sight of blood or needles.
- Prior administration of immunodepressant or corticosteroids, antianaphylaxis treatment, cytotoxic treatment in last 6 months
- Prior administration of other research medicines in last 1 month
- Prior administration of attenuated vaccine(s) in the last one month
- Prior administration of inactivated vaccine(s) in the last 14 days

- Any condition that in the opinion of the investigators may interfere with the participants' compliance or evaluation of study objectives

8.4. Withdraw from the study

The participants have the right to withdraw from the study at any time during the study period, and the investigator should record and handle the following cases:

- Before the termination because of loss of contact.
- Request from participants without any reasons.
- Reasons unrelated to the study requirements should be recorded for the withdrawal such as go out for a long time, moved.
- Reasons related to the study requirements should be recorded, such as the inability to tolerate the adverse reactions, endure biological specimen collection. AE/SAE should be solved by investigator.
- Early termination of the study can be advocated by participants, including stopping vaccination, biological samples acquisition and safety otherwise data before withdrawal can be used to analysis. If the participants banned the investigators to continue using all the relevant research issues, the research information will not be used for analysis.
- Part of early termination of the study can be advocated by participants such as stopping vaccination, biological samples acquisition while other researches continue.

8.5. Termination of study

8.5.1. Completed safety observation

Safety of 28 days after immunization and the SAE reports in entire study period are completed.

8.5.2. Completed immunogenicity research

The participants meet the inclusion and exclusion criteria participated in randomization, vaccination, processing the blood collection at day 0, 14, 28 and 168 (month 6).

9. METHODS AND PROCEDURES

9.1. Participants screening

Healthy local 18-50 years old residents in Sierra Leone will be informed about the study and approved consent sought from them to be a volunteer. After physical examination and screening according to the inclusion and exclusion criteria, they will be eligible to participate in this study. Before they sign the informed consent, they have enough time to think about it, and a withdrawn at any time during the trial is permitted.

Following operation will be performed during the selection:

- Demographic data.
- Physical examination, including HIV test and urine pregnancy test (female only).
- History of disease.
- Conforming to the inclusion criteria and not the exclusion criteria.

9.1.1. Laboratory examination

9.1.1.1. Urine pregnancy test

Pregnancy test will be performed on target women of childbearing age (18-50 years old) for early pregnancy detection.

9.1.1.2. HIV antibody screening

HIV antibody will be screened for all the volunteers. Only the HIV specific antibody negative volunteers could be enrolled in the group.

9.2. Randomization and Blinding

9.2.1. Randomization and blinding method

Randomization list will be generated by independent statistician using SAS software. The independent statistician will generate the randomization list of 500 random codes, randomly allocated these codes to the high dose vaccine, or low dose vaccine, or placebo in a ratio of 2:1:1, using a “Proc plan” procedure in SAS by block randomization. Each person dose of investigational vaccines is assigned

a code from a randomization list. The package of the investigational vaccine (including high dose vaccine, low dose vaccine and placebo) will be relabeled, using identical package with a randomized code which is the unique identifier for each person dose. Therefore, all the vaccine and placebo doses are blindly coded. The persons who participate in randomization and blinding must not participate in any other process of the clinical trial, also must not disclose the contents of blinding to any personnel. Eligible participants enrolled into this study, will be assigned a sequential number according to their sequence of enrolment and received vaccine or placebo labelled with the same numbers. Thus, participants are randomly assigned to receive high dose vaccine, or low dose vaccine, or placebo in a ratio of 2:1:1.

The independent statistician should produce a copy of emergency blinding code which should note the title of program and the character “To Be Opened Only in Case of Emergency”. Personnel who involved in randomization and blinding are not allowed to participate in any other procedure in this study.

Blinding will be maintained for all participants and investigators and their study staff participating in this study.

9.2.2. Method of bind breaking and unblinding

When the last participant completed the 28 day visit, CRF completion, a database entry and blind state audit should be completed. Un-blinding should be after the publication database lock statement when all parties involved in such as the sponsor, investigator, statistical representatives.

During the study, the breaking of the treatment code is forbidden, except in the event of a medical emergency when the investigator believes it is necessary to determine the treatment code in order to initiate appropriate treatment. If knowledge of the treatment code is required, the investigator will open only the specific participant’s code envelope. A signature, date, time and reason will be written on the opened code envelope and the participant with this code has to be withdrawn from this study.

The investigator will assess the relationship between the adverse event and the investigational products before the treatment code is un-blinded. The investigator will immediately notify the sponsors at the 24 hours emergency call number (Lihua Hou +23276551560) when the treatment code blinding is broken on any participant for any reason during the study. The reason for the blinding being broken

must also be documented in the participant's medical records and in the CRF.

At the end of the study, all code envelopes (intact and opened) must be accounted for and are to be collected by the monitor to be destroyed.

9.2.3. Blind maintenance

In the process of the blinding, the staffs responsible for preparing the vaccine are non-blind person, who know distribution of participants because the vaccine group A (high dose group) and vaccine group B (low dose group) need different vaccine preparation methods (see 9.3.2).

Besides the staffs for vaccine preparation, all other researchers do not know the treatment allocation of participants. The two un-blinded staffs only responsible for the vaccine preparation, are not allowed to do any records or tell any person the distribution of participants or participate in the test of any other work (including follow-up observations).

After the visit of 28 days after vaccination, the data will be checked by the statistical part and then locked for the interim analysis. The data will be unblinded. Serious adverse event after day 28 and final visit at month 6 will be opened. But the staffs responsible for the laboratory tests will be kept in blinded during the whole study, including the serum tests at month 6.

9.3. Vaccine inoculation

9.3.1. Experimental vaccine Ad5-EBOV

Experimental vaccine Ad5-EBOV, developed by Beijing Institute of Biotechnology and Tianjin CanSino Biotechnology Inc., is a replication defective Adenovirus Type 5 Vector based vaccine which expresses Ebola virus Zaire (Guinea, 2014) envelope glycoprotein. The final product is lyophilized white powder, with 4.0×10^{10}vp/vial. The antigen contents in the Ad5-EBOV were measured according to Ebola vaccine antigen standard by the National Institute for Food and Drug Control (NIFDC).

The placebo is lyophilized-dried powder, contains same formulation of excipients as vaccine, with no virus particle which could express Ebola virus Zaire envelope glycoprotein. The placebo was also measured to be qualified by NIFDC.

The sterile water for injection is developed by SINOPHARM GROUP CO. LTD. (Lot: 1405544-All), 2ml per vial. The expire date is 2017, 04,

9.3.2. Administration

One injection will be allocated intramuscularly in the arm at day 0.

Experimental vaccine group A (4×10^{10}vp/vial, 4 vials): 1 ml sterilization injection water per dose to dilute 2 vials (4×10^{10} vp/vial), one shot in each arm, total dose of 1.6×10^{11}vp. The inoculation site was the central lateral deltoid in the upper arm, and inoculation route was intramuscular injection

Experimental vaccine group B (4×10^{10}vp/vial, 2 vials): 1 ml sterilization injection water per dose to dilute 1 vial (4×10^{10}vp/vial), total dose of 8×10^{10}vp, one shot in each arm. The inoculation site was the central lateral deltoid in the upper arm, and inoculation route was intramuscular injection

Placebo group (0 vp/ vial, 2 vials):1 ml sterilization injection water per dose to dilute 1 vial, total dose of 0 vp. The inoculation site was the central lateral deltoid in the upper arm, and inoculation route was intramuscular injection

Before injection, 75% alcohol is used for disinfection at the injection site and intramuscular vaccination will be administrated several minutes later. Shaking the vaccine before use and make vaccine fully dissolved in sterile water for injection. No intravascular, intradermal or subcutaneous injection is allowed with the investigational vaccine. During the vaccination and 60-minute safety surveillance after vaccination, appropriate emergency medical equipment and doctors should put on standby in case that possible allergic reaction after injection.

Only the qualified doctors, Community Health Officer (CHO) or qualified nurse will give the injection. For the purpose of vaccine/placebo administration, a medically qualified member of the study staff is a study physician or a study nurse, as applicable to the investigator's local practice.

9.3.3. Vaccine management

The sponsor should provide the full amount of vaccine, including the alternate vaccine.

Test vaccine packaging must comply with the requirements of clinical trials.

Sponsor is responsible for transportation of the test vaccine to the research center, vaccine transportation temperature records (in accordance with the vaccine cold chain temperature) should be submitted along with the experimental vaccine together to investigators. The blind file, emergency blind file and inspection reports (qualified) should be submitted with the sponsor and manager in research center.

Special area should be used to store and lock the test vaccine to get rid of unauthorized persons. Vaccine is forbidden to inject other ones except participants.

The temperature of the monitoring instrument, transport and storage of the vaccine should be daily monitored (am and pm manually). Once the temperature deviation happened, as the temperature beyond the provisions of the range of 2-8, the investigators and sponsors should be immediately informed, and the "cold chain damage report form" should be filled in, too. The temperature-deviated vaccine should be identified, placed separately and suspended. Continual usage of vaccines must be written approved by Biotechnology Institute of Academy of Military Medical Sciences/Tianjin CanSino Biotechnology Inc. Vaccine out of requirements should be on-site sequestration.

The test vaccine should be stored in a refrigerator or freezer and cold chain equipment is equipped with a thermometer with the vaccine administrator records temperature every 15 minutes.

Vaccine administrators release the test vaccine to the vaccination staff according to number of participants and vaccine. The left test vaccine packing should be recycled after inoculation and detailed records of test vaccine and recycling packaging are needed

At the end of vaccination, vaccine and package of the vaccine will be checked and recycling stored by administrator. At the end of the study, the researchers will check all the remaining vaccine and package and deliver them back to sponsors.

At any time, the total number of vaccines, unused or damaged vaccines must be consistent with the applicants provided, otherwise, description is needed to be provided by investigator.

9.3.4. Alternate vaccine

500 person-dose of blind packing experimental vaccine should be provided by sponsors. Another 30 person-dose of experimental vaccine A, experimental vaccine B and placebo, respectively should be provided in case of vaccine damage or other unusable situation. If vaccine is damaged, the non-blind physicians could replace vaccine according to the corresponding category of participant. Although there's no need immediately noticing the sponsor in this case (except cold chain accident), the investigator should have vaccine damage and replacement record in details.

9.3.5. Combined medication/vaccine

When the medical events happen during the study period, the participant are allowed to carry out the appropriate medical treatment, but the medical treatment should be recorded in time.

Other vaccination is not recommended except for emergency during the research period, such as rabies vaccine, tetanus vaccine, or other emergent vaccination need. Any vaccine used is required to be recorded during the study period.

9.4. Safety follow-up

9.4.1. Methods of safety observation

After vaccination, each participant will be asked to stay at study site for at least 60 mins safety surveillance. Doctors will monitor the general safety condition of the participants and teach them to observe any adverse reactions or events on the diary card. Doctors will teach them how to use a digital thermometer to measure the temperature. If there is no significant adverse reactions, participants will be allowed to go home and report auxiliary temperature and any adverse events (AEs) for seven consecutive days. Each participant will get a digital thermometer, a measure ruler and a cell phone before he or she leave the site for reaction measurement and report.

During the first 7 days after vaccination, several trained staffs will collect the safety data from participants daily by phone or home visit. These trained staffs will record the temperature and any adverse event reported by participants on the Diary Card. If the symptom of reaction is severe and needs medical intervention, the trained staffs must instruct the participants to seek the treatment in community clinics, and record the treatment on the Diary Card.

From day 8 to the end of the study, no need to collect the adverse event actively. But once a serious adverse event is reported from subject, the staff will immediately inform the principal investigator by telephone. And assist with the serious adverse investigation.

9.4.2. Safety observation and grade of adverse reaction/event

9.4.2.1. Definition of adverse event and serious adverse event

An adverse event (AE) is any untoward medical occurrence in a participant administered an

investigational product and which does not necessarily have a causal relationship with this treatment. A serious adverse event/reaction (SAE) is occurrence of any untoward medical during the whole study period that:

- Results in death.
- Is life-threatening (an event in which the participant is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe).
- Results in persistent or significant disability/incapacity.
- Requires hospitalization or prolongation of an existing hospitalization.
- Is a congenital anomaly/birth defect.

In addition, medical and scientific judgment will be exercised in deciding whether other conditions will also be considered serious, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant's safety or may require intervention to prevent one of the other outcomes listed in the definition above. These will also be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

9.4.2.2. Grading for adverse events

All adverse events will be graded according to "The standard guidelines for adverse reactions grading of vaccine clinical trials" issued by China state Food and Drug Administration (SFDA). The presence of solicited and unsolicited adverse events and any serious adverse events will be described in terms of the incidence, intensity and relation to vaccination. The incidence of adverse events will be based on the most severe response, and expressed in terms of the number and proportion of individuals who had adverse events in each group.

Injection site

Pain (at the injection site)

Grade 1(mild)= Mild pain when the injection site was touched

Grade 2(moderate)= Moderate pain when the injection site was touched

Grade 3(severe) = Severe pain, refuse to be touched at the injection site

Grade 4(potentially life threatening) = Need emergency treatment or hospitalization

Induration (at the injection site)

Grade 1 (mild) = Diameter : <15mm

Grade 2 (moderate) = Diameter :15～30mm

Grade 3(severe)= diameter >30 mm- ≤ 50 mm

Grade 4 (potentially life threatening)= Gangrene

Redness (at the injection site)

Grade 1 (mild) = Diameter : <15mm

Grade 2 (moderate) = Diameter : 15～30mm

Grade 3(severe)= Diameter : >30mm

Grade 4(potentially life threatening)= Gangrene

Swelling (at the injection site)

Grade 1 (mild) = Diameter : <15mm, with no limited movement

Grade 2 (moderate) = Diameter :15～30mm; with limited movement

Grade 3(severe)= Diameter :>30mm; with severely limited movement

Grade 4(potentially life threatening) = Gangrene

Rash (at the injection site)

Grade 1 (mild) = Diameter : <15mm

Grade 2 (moderate) = Diameter :15～30mm

Grade 3(severe)= Diameter :＞30mm

Itch (at the injection site)

Grade 1 (mild) = Mild itch at injection site

Grade 2 (moderate) = Moderate itch on injected arm

Grade 3(severe)= Severe itch all over the body

Systemic reactions

Fever (axillary temperature)

Grade 1(mild) = ≥ 37.1°C≤ 37.5°C

Grade 2(moderate) = ≥ 37.6°C ≤ 39.0°C

Grade 3(severe) =＞39.0°C

Allergic reactions

Grade 1(mild) = Itch without rash

Grade 2(moderate) = Localized urticaria

Grade 3(severe) = Generalized/extensive urticaria, angioedema

Grade 4(potentially life threatening) = Severe allergic reactions

Headache

Grade 1(mild) = Mild, with no interfere with daily activities

Grade 2(moderate) = Moderate, with a mild impact on daily activities

Grade 3(severe) = s Severe, with a significant impact on daily activities, need a treatment

Grade 4(potentially life threatening) = Stubborn headache, need an emergency treatment or hospitalization

Fatigue

Grade 1(mild) = Mild, with no interfere with daily activities, last no more than 48 hours

Grade 2(moderate) = Moderate, with a mild impact on daily activities, last over 48 hours

Grade 3 (severe) = Severe, with a significant impact on daily activities, can't work

Grade 4 (potentially life threatening) = Unable to do anything, need an emergency treatment or hospitalization

Nausea /Vomiting:

Grade 1(mild) = 1～2 episodes per 24 hours, with no interfere with eating and drinking

Grade 2(moderate) = 2～5 episodes per 24 hours, with a mild impact on eating and drinking

Grade 3(severe) = >6 episodes per 24 hours, with a significant impact on eating and drinking, need a treatment

Grade 4(potentially life threatening) = Need an emergency treatment or hospitalization

Diarrhea:

Grade 1(mild) = Mild, 2～3 loose stools per day or mild diarrhea last <1 week

Grade 2(moderate) = Moderate, 4～5 loose stools per day, diarrhea last >1 week

Grade 3(severe) = Severe, >6 loose stools per day, or bloody stool, electrolyte imbalances, need a treatment

Grade 4(potentially life threatening) = Need an emergency treatment or hospitalization

Muscle pain

Grade 1(mild) = Mild, with no interfere with daily activities

Grade 2(moderate) = Moderate, with a mild impact on daily activities

Grade 3(severe) = Severe, with a significant impact on daily activities, need a treatment

Grade 4(potentially life threatening) = Need an emergency treatment or hospitalization

Joint pain

Grade 1(mild) = Mild, with no interfere with daily activities

Grade 2(moderate) = Moderate, with a mild impact on daily activities

Grade 3(severe) = Severe, with a significant impact on daily activities, need a treatment

Throat pain

Grade 1(mild) = Mild, with no interfere with daily activities

Grade 2(moderate) = Moderate, with a mild impact on daily activities

Grade 3(severe) = Severe, with a significant impact on daily activities, need a treatment

Cough

Grade 1(mild) = Transient, need no treatment

Grade 2(moderate) = Persistent cough, resolved after treatment

Grade 3(severe) = Severe cough, not resolved after treatment

Grade 4(potentially life threatening) = Need an emergency treatment or hospitalization

The general grading principles for other adverse reactions (the clinical abnormalities that were not involved in the above classification):

Grade1 (mild) = Mild, with no interfere with daily activities

Grade 2 (moderate) = Moderate, with a mild impact on daily activities

Grade 3 (severe) = Severe, with a significant impact on daily activities, need a treatment

9.4.3. Outcome of AE

The adverse reaction / event outcomes include:

- Recovery
- Not yet recovered

- Recovered but sequelae
- Death
- Loss of visit

9.4.4. Relationship between AE and vaccine

Researchers should make the best interpretation of AE, and assess the possible causal relationship between vaccine inoculation and alternative reasons (such as history of underlying diseases, combined treatment of causation). This applies to all AE, including severe and non-severe.

The assessment of causality will be reasonably explained in the following or more aspects of the event:

- The similar reaction to the solution was observed in the past;
- identical events of similar types solution have been reported in the literature;
- the incident occurred along with the time of the vaccination, and again after the secondary vaccination

According to definitions, all the collected AE (that is, the local adverse event of the collection of the report) will be considered to be related to vaccination.

The causal relationship of AE should be evaluated according to the following questions, and according to your judgment, the reasonable possibility of relationship between AE and vaccination is caused by the vaccination:

- Related: there is a suspicion that a link between vaccine and the AE (do not need to be determined); the vaccine has a reasonable potential for promoting the AE.
- Unrelated: there is no suspicion that a link exists between vaccine and the AE; there are other more likely causes, and vaccination has not been suspected to promote the AE.

9.4.5. Reporting of SAEs

Any serious adverse event, including death due to any cause, which occurs during this study, whether or not related to the investigational products, must be reported immediately. The un-blinding of single cases by investigators in the course of the clinical trial will only be performed if relevant for the safety of the participant.

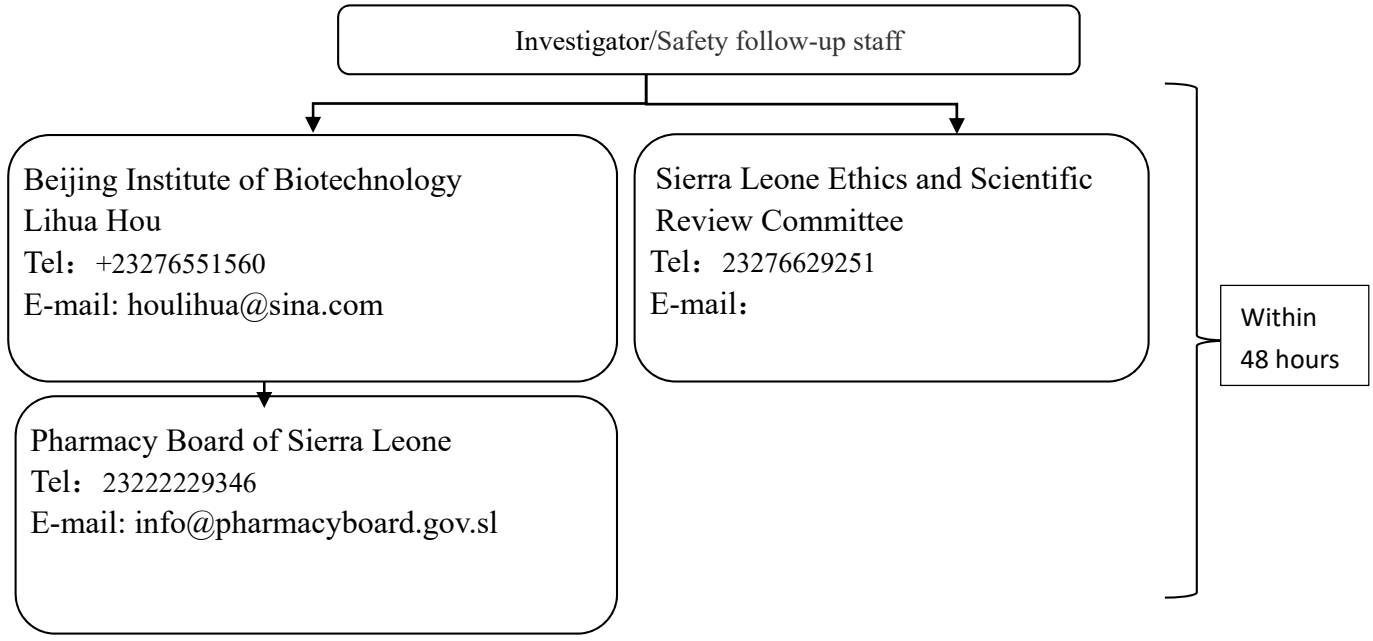
The SAE report form must be filled and submitted to Pharmacy Board of Sierra Leone and the

Sierra Leone Ethics and Scientific Review Committee within 48 hours of the first knowing about the occurrence of the event. All SAEs will be recorded on the case report form and source documents.

In case of death of a participant post-vaccination, and may be correlated with the vaccination, verbal autopsy must be carried out as per WHO Gudelines using "The 2014 WHO verbal autopsy instrument" (Appendix 12,http://www.who.int/healthinfo/statistics/verbalautopsystandards/en/). A copy of autopsy report should be attached with the SAE form.

All suspected adverse reactions related to an investigational products, both in unexpected and serious (SUSARs) are expedited reporting to investigator. Also post-study SUSARs that occur after the patient has completed a clinical trial and are reported by an investigator to the sponsor, qualify for expedited reporting.

The sponsor is responsible for the prompt notification to all concerned investigators, the fatal or life-threatening SUSARs will be notified by the sponsor to the Pharmacy Board of Sierra Leone as soon as possible but no later than seven calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. All other SUSARs and safety issues deserving expedited reporting must be reported to the competent authority and -dependent on national provisions - to pharmaceutical supervision department in Sierra Leone as soon as possible but no later than 15 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. The SAEs/ SUSARs report procedure should as follows:



Sponsor will also be responsible for reporting the SAEs to the DSMB for safety review. The fatal or

life-threatening SUSARs no later than seven calendar days. Other SUSARs no later than 15 calendar days.

9.4.6. Reporting of the occurrence of new HIV infection or pregnancy

The participants are asked to report new diagnosed HIV infection or pregnancy to the investigator or safety follow-up staffs. The report procedure shall follow the SAE report procedure and using SAE report form. The occurrence of new HIV infection also need to be reported to the DMSB for safety evaluation. Any pregnancy after vaccination during the study period will be monitored/followed up till delivery and 28 days after delivery of a normal baby. If delivery baby with abnormality, the cause of the abnormality will be investigated by the DSMB.

9.4.7. Record of safety observation

Any clinically meaningful adverse event occurred after vaccination should be recorded in the diary card. Verification and medical visits by investigator respond to adverse events are required, such as investigation of medical history, physical examination and necessary laboratory examination (if required). Participants should receive appropriate medical treatment until the adverse event decline completed with complete records.

The record of adverse events should include the following:

- Description of adverse events
- Start and end time of adverse events
- Severity (grade)
- Relationship with vaccination
- Laboratory findings
- Treatment measures
- Outcome

If there are allergies, SAE, or a level 3 adverse events or above happening in safety observation period, medical treatment should be provided until symptoms disappeared or stabilization of symptoms. Record the details of visit exactly.

9.4.8. Medical treatment of AE

If the participants received local or systemic adverse reactions or events or serious adverse events, researchers should provide appropriate treatment or medical consultation to reduce or remove suffering. The medical treatment of green channel could be started if it is necessary. The medical procedures and outcome should be exactly recorded.

9.5. Laboratory assay and immunogenicity assessments

9.5.1. Zaire Ebola virus GP-specific antibody responses

9.5.1.1. Time point

Serum antibody titers against the Zaire Ebola virus GP will be determined at day 0 (immediately before vaccination), day 14, day 28, and day 168 (month 6).

9.5.1.2. Blood sample processing and detection methods

The blood sample shall be isolated for serum, and the isolated serum from each participants shall be collected into several sterile tubes, 1ml in each, and store in cryogenic refrigerator below -20℃。
Zaire type of Ebola virus antibodies will be detected by ELISA.

9.5.1.3. Evaluation

The level of antibody to Zaire glycoprotein after immunization was the primary evaluation measurements of the immunogenicity in serum. The antibody responses in different time points will be compared between vaccine group and placebo group.

9.5.2. Neutralizing antibody titers response to Ad5

9.5.2.1. Time point

Neutralizing antibody titers response to human Ad5 will be detected at day 0, day 14, day 28 and day 168 (month 6).

9.5.2.2. Blood sample processing and detection methods

The isolated serum will be used for the Neutralizing antibody titers response to human Ad5 assays. Laboratory staffs will detect neutralizing antibody in serum by use of replication defective human type 5 adenovirus expressing luciferase (Ad5-luc).

9.5.2.3. Evaluation

The neutralizing antibody titers against human Ad5 pre-vaccination and the post-vaccination will be compared to reveal the difference. The relationship between the pre-existing antibody titers against Ad5 and the specific immune response against Zaire Ebola virus (including both humoral and cellular responses) will be explored.

9.5.3. Detection of HIV

9.5.3.1. Time point

HIV infection will be detected at day 0 and day 168 (month 6). Two drop of finger blood will be collected for each HIV test.

9.5.3.2. Evaluation

Participants with negative response to HIV infection on day 0 could join into the group. The HIV infection incidence of the participants during the study will be compared with the average HIV infection incidence in local area. And the HIV infection incidence in the vaccine group and placebo group will be compared.

9.6. Data management

9.6.1. Source documents and source data

The purpose of source documents is to record the existence of the participant and substantiate the integrity of the trial data collected. The Investigator must maintain the trial source documents accurate,

complete, legible and up to date.

Examples of source documents are: participant screening, laboratory measure reports, enrolment log, participant's diary cards, hospital records, informed consent forms, investigational dispensing and reconciliation forms, participant's file and records kept at the pharmacy or at the laboratories, mail, certified letters.

Source data are the data contained in source documents (originals or certified copies). The investigator is responsible for the accuracy and completeness of the data reported in source documents. Data reported in the CRFs that are derived from source documents should be consistent with source documents and any discrepancies should be explained.

All CRFs must be signed by the Investigator. Incorrect data must be crossed-out with a single line, then initialed and dated. Correction fluid or similar corrective methods that mask the original data will not to be used. These rules also apply to the completion of SAE Reporting Forms, Data Correction Forms, and ICFs.

9.6.2. Clinical data management

The investigators should fill in all case report forms (CRF). CRF is used to record data in clinical trials, is an important part of clinical trials and research reports, filling shall be clear and intact, and also should be completed with Chinese language and black pen. Only authorized investigators could correct the errors in the CRFs, The original record can't be obliterated or overwrite. Investigator should draw a horizontal line across the original data which should be corrected, and specify the corrected data in the space next to them, and noted the signature and date.

According to the project requirements, the data collection, biological sample collection and examination should be done in the visit window, the original documents and records shall be complete and the results of the examination also should be timely entered into case report form (CRF).

Auditors should conduct regular and irregular audits of data records until CRF are completed, auditors should carefully verify CRF number of the participants, the number of pages in each CRF and necessary signatures of researchers. The main contents of audits should be focused on signed informed consent, volunteer screening into the group, vaccination, management of the investigational vaccine, safety observation and immunogenicity of specimen collection and preservation, Consistency between research

data and the original data is the emphasis of audits. Manual verification results shall be recorded. Transfer of CRF research data should be documented. For each batch of data, double entry, quality control and triggers to computerized logic and/or consistency checks will be systematically applied in order to detect errors or omissions. Queries will be generated and submitted through Data Clarification Forms to the Investigator for resolution.

9.6.3. Database creation and data entry

－ Database creation

The person, who is responsible for data management, establish the database structure and inspection procedures to ensure that the database can be correctly converted to SAS file format, and the database structure can also be modified and confirmed by tentative data entry.

－ Verification of CRF

Before CRF entry, data management staff will carry out the CRF verification again, mainly to see if there are obvious errors and omissions.

－ Data entry

Data entry staff will start data entry after several training, double entry will be accepted.

－ Comparison and check of data

The consistency check of the database that be independently completed by two persons will be executed. Inconsistent values and information should be reported. After that, the raw data will be checked one by one and the mistake will be corrected until database is left in a consistent state. The computer program that has been prepared and confirmed will be applied to do logical consistency check. Before modifying the database, Query table should be shown and confirmed by researchers until there is no question. A certain proportion of CRF will be randomly selected to finish quality control, and to compare with the data in the database, to ensure that the data in the database and the CRF content are consistent.

9.6.4. The database lock

Blind review of the database is required before Statistical analysis. The aim of blind review is to determine the population that will be analyzed according the evaluation criteria, including full analysis

set (FAS) under the Principle of ITT (intention to treat analysis) , per-protocol set (PPS) and safety analyzes data set, confirmation of the deviation from the project and other influences on database. Database will be locked after blind review be confirmed.

9.7. Statistics plan and statistical analysis

9.7.1. Sample size calculation

According to the results of Phase I clinical trial in Jiangsu, we assume that the positive rate of low-dose and high-dose vaccine groups was 85% and 95% respectively and placebo no more than 5%. The allocation ratio will be set as (high-dose: low-dose: placebo)=2:1:1. In order to assure at least 80% of certainty to identify the difference when the lower dose is compared with the higher dose at α value is 0.05, at least 197 participants in high-dose vaccine group and 99 participants in low-dose vaccine group are needed. Around 15% visit loss rate is assumed so we set the sample size as 250 people in high-dose vaccine group, 125 people in low-dose and placebo group, respectively. The total sample size is 500 people.

9.7.2. Interim analysis

After the last participant complete the Visit 3 (28 days after vaccination), the data of the clinical trials from Visit 1 to Visit 3, will be entered into the database for blind review. Then database (Visit 1 to 3) is locked, interim analysis will be done by the independent statistical party. Statistical party must not divulge blinded code. The clinical trial sites still remain in blinded.

9.7.3. The final analysis

After the last participant complete the Visit 4 (168 days after vaccination), database entry is completed, blind review is finished and database is locked, the final analysis will be done by the independent statistical party.

9.7.4. Analyzed data sets definition

9.7.4.1. Data set for safety evaluation

All randomized participants who received vaccination should be included in the safety evaluation. Thus, the safety analysis will be performed on the basis of (Intention-To-Treat) ITT cohort.

Events will be reported on per-individual basis, i.e. counting individuals rather than events. This means that even if a participant suffered a same event repeatedly during the follow-up, the event will be counted only once, except for SAEs. Repeated same adverse events in participant will be summarized according to the following rule: if a participant suffered the same adverse event more than once, the event will be assigned the worst severity, the closest relationship to the vaccination and the earliest starting date. In the listings, however, all occurrences of the adverse events will be shown.

9.7.4.2. Data set for immunogenicity evaluation

Full analysis set (FAS) for immunogenicity analysis: FAS is based on ITT (intention to treat analysis) principle to determine the participants. All of the participants that meet the inclusion / exclusion criteria, randomization, receiving vaccination, and have at least one blood testing result after vaccination ,were included in the FAS set for immunogenicity.

Per-protocol set (PPS): It is a subset of FAS. In this set, all participants that meet the inclusion / exclusion criteria and complete the vaccine inoculation within visit window according to the protocol, and complete the blood collection on day 0, 14 and 28, with no significant deviation or violation of protocol.

In this study, the FAS are the primary analysis set for immunogenicity evaluation, but the PPS will also be analyzed at the same time. Any difference of analysis results existed between PPS and FAS, will be discussed in the report.

9.7.5. Statistical methods

9.7.5.1. Safety analysis methods

Safety analysis of this experiment is mainly descriptive analysis of incidence rate of adverse reaction

or adverse events. A chi-square test can be used to compare the proportion of participants with adverse reactions in different groups, Fisher's exact test will be used when it is necessary.

9.7.5.2. Immunogenicity analysis methods

Analysis of immunogenicity indicators on antibody levels need to do logarithmic transformation, the results of analysis should be shown in GMT, standard deviation, median, minimum and maximum values and 95% confidence intervals. ANOVA will be used to compare the GMTs among different groups, and then SNK methods will be used for further pairwise multi-comparison. Chi-square test can be used to compare categorical indicators between groups such as positive conversion rate of immune response, if it is necessary, Fisher's exact test will be used. Statistical analysis method of repeated measures data can be used to analyze experimental data at different time points in this study.

SAS software was used for all analyses, test statistics and the corresponding p values are given. All statistical tests were two-sided and significance was set at $P \leqslant 0.05$ (more detailed information, please read the reference the statistical analysis plan (SAP)).

9.7.6. Statistical Considerations for the DSMB

All the SAEs report along with the HIV infection report will be submitted to the DSMB. The DSMB will follow the SAE reports and determine the relationship with the vaccination of the investigational vaccines. If a SAE is considered to be “may be associated with vaccination”, or the reported incidence of new HIV infection is excess 2.5%, then the DSMB may unblind the reported cases, and re-estimate the risk of participants. If necessary, the DSMB discuss with sponsor and investigator about the risk of participants in this study which may resulting in a pause or an early termination of the trial. If a fatal SAE is considered “to be definitely associated with vaccination” or the reported incidence of new HIV infection is excess 5%, then the DSMB shall unblind the reported cases and call an early termination of the trial if necessary.

10. CLINICAL MONITORING AND CONTROLLING OF EXPERIMENTS

10.1. Responsibility

Quality assurance system is maintained by sponsor to ensure that the research is conducted. The data collection, records and reports should be complied with the requirements of the GCP and protocol. The protocol of clinical trial and all relevant procedures should be fully comprehended by investigator and monitor including experimental vaccine information, obtain informed consent procedures, reporting procedures of adverse events (including serious adverse events) and the CRF program completion.

The main investigators should have a clear mandate for the division and management of all the researchers involved in clinical trials and should develop SOP for all research positions.

The personal data of the participants should be kept confidentially by investigators. CRF or other documents provided to the applicant shall be identified only through code or random numbers. The participants' identification list and the selection of the registration form (including the full name, age and address) are saved by the investigators. According to the GCP principle, the original data of each participant is allowed to be monitored, inspected by administration department.

The monitoring should be carried out according to the laws of a certain time. The consistence of original data and information in CRF will be checked to assure accuracy and the completion. If CRF and original data are inconsistent or not timely completion, urging to investigators is required as soon as possible. The monitor will evaluate the informed consent process, vaccine transportation storage and the progress of the documents. Compliance to protocol will be examined to observe procedure and discuss some issues with investigators. There must be monitoring records. After the study, the monitor shall provide a copy of the audit record to the sponsor.

10.2. Quality control of experimental vaccine

Experimental vaccines should be managed specifically. The vaccine management and recording system should be available from sponsor to investigator and accept the supervision of the monitor. The number of vaccines, people vaccinated, remaining quantities and the received amount of damage need to be recorded in the work log.

The sponsor will send the experimental vaccine to the investigators once a time. When the investigators

found that damaged package of the vaccine, vaccine modification or the bulk material cannot be shaken to dissolve, the experimental vaccine will be returned to the sponsor without use. If the transportation and preservation process in cold chain system was damaged, the vaccine should not be used. They should be separately stored and clearly marked and returned to the sponsor by the responsible person for management. Investigators must sign the vaccine transfer receipt to confirm all vaccines received, the receipt shall be stated briefly the information of received vaccine including the amount, the package, cold chain system.

The left test vaccine packing should be recycled after inoculation and detailed records of test vaccine and recycling packaging are needed

At the end of vaccination, vaccine and package of the vaccine will be checked and recycling stored by administrator. At the end of the study, the researchers will check all the remaining vaccine and package and deliver them back to sponsors. The total number of vaccines, unused or damaged vaccines must be consistent with the applicants provided, otherwise, description is needed to provide by investigators.

10.3. Controlling of files

10.3.1. Original files

Original data includes the participants' demographic data, inquiry results of medical history, examination results, laboratory test results, vaccine immunization records, records of bleed, combined medication and adverse events / reaction and treatment and outcome etc. All information shall be recorded in the original medical records, and kept in a special room. The original data will be archived in the research center, and it is the basis of data authenticity and integrity.

Visit recording and other original records should be carefully, accurately and immediately filled by investigators. All the raw data should be collected in the record of inoculation and visit. The raw records include the following basic data:

Items of experiments, participants' number, random coding of participants

Demographic data

Inclusion / exclusion criteria

Physical examination results

Laboratory test results (including Immunology)

Vaccination record

The date of the visit and the date of termination of clinical trial

Adverse events /reactions and their treatment and outcome

Blood collection record

Concomitant drug treatment, medical treatment and other vaccination

10.3.2. Case report form

Two copies of carbonless CRF are provided for every participant. The first page of CRF will be saved by the sponsors, and the second will be preserved by the investigators. Only investigators and approved staff are allowed to visit CRF during the trial.

Whether the participants complete the research or withdraw, accurate data must be signed on the CRF by investigators. The cause of the early termination should be mentioned in CRF.

The situation of each stage of the participants should be reflected in CRF during the trial. Names of the participants cannot be shown in CRF, the appropriate code or the names in initials could be used.

All the data on the CRF comes from the raw data and will be consistent with the original data. All the data recorded in the CRF should be recorded in the original data.

Written documents should be issued after modified by the sponsors, investigators and other relevant parts about clinical trials meetings, protocol, informed consent and all the original data, and all their agreement documents will be copied in two files and saved respectively.

10.3.3. Storage of files

Preservation of clinical trial data must be accorded to GCP. Investigators should save data at least 5 years more than the end of clinical trials while the clinical trial data should be permanently preserved by sponsors and Ministry of Health & Sanitation of Sierra Leone.

10.4. Quality control of biological sample

Serum samples for antibody detection should be collected within 5 hours after centrifugation with a hemolysis rate of serum less than 2% and the error rate less than 1%.

The serum samples used for other detection are collected, processed and preserved in strict accordance

with the requirements of SOPs.

10.5. Ownership and publication

All data /information generated in the research center (except the medical records of the participants) belong to sponsors and Ministry of Health and Sanitation, Sierra Leone. If the written contract confidentiality terms of this study should be offset with this statement, processed by prevail of this statement.

Before the research results in submission, speaking, teaching or other form of public (collectively referred to as "publication"), a content copy must be submitted to sponsors to obtain written approval, and the results can be published. The confidential information and personal information of the participants (such as the name or initials) cannot be included in research results.

10.6. Confidential

The sponsor, investigators, ethics committee (IEC) or representatives of full authorized management such as Pharmacy Board of Sierra Leone, China FDA have the right to access the clinical trial data, but the relevant content cannot be used for any other clinical trials or disclosed to any other person or entity.

A confidentiality agreement must be signed by the investigators to verify their awareness and agreement with the information in this research is kept confidential.

The investigators and other researchers should keep all the information provided by the sponsors and all the data / information generated in the research center (except the medical records of the subjects) confidential. This information and data cannot be used for any other purpose out of this study. This restriction does not apply to: (1) research information is publicly but not due to the violation of investigators and researchers; (2) public the research information to the IRB / IEC for the purpose of evaluation; (3) to provide proper medical assistance lead to information disclosure; or (4) research results published after sponsor authorized. If the written contract confidentiality terms of this study should be offset with this statement, processed by prevail of this statement.

11. Time-table

This study will last 14 months from the preparation before the study to the completion of the final summary report, and the clinical trial schedule is shown in the following table (for reference only):

Clinical trial schedule	Estimated time
1.Preparation before the study	2 months
2.Reviewed and Approved by Ethics Committee and Pharmacy Board of Sierra Leone	3 months
3.The first subject recruited into the group	1 month
4.The last subject finish Visit 4	6 months
5.Final Analysis	1 month
6.Summary Report	1 month

12. The Ethics Committee approval

12.1. Ethical review and approval

The Principal investigator should submit the clinical trial protocol and all necessary appendix documents to The Ethics Committee for the initial review as required, including but not limit to the following documents:

- Clinical Trial Protocol (indicate the version number/date)
- Informed Consent (indicate the version number/date)
- Case Report Form (indicate the version number/date)Diary Card (indicate the version number/date)
- Investigator's Brochure
- Primary Investigator's CV

The certificate of approval should be issued to the investigator after getting the approval of the ethics committee. The investigator should submit a copy of the certificate of approval to the sponsor.

12.2. Follow-up Auditing

To audit the method of participant recruitment, if the information offered to the subjects or impartial witness was completed, understandable; if the informed consent was offered appropriately, if the SAE was reported in time. If there was SAE occurred on the subjects, they could get immediate medical treatment.

During the research period, the Ethics Committee should monitor that if the ratio of risk and benefit increased and if the participants' rights and interests are effectively protected.

12.3. Potential danger and danger minimization

12.3.1. Benefit and Risk

The subjects/participants in this study will not pay for the Ad5-EBOV vaccines and will obtain the reasonable transportation expenses, lost income, blood donation, and nutrition fee compensation. The subjects will get one vaccination with Ad5-EBOV or placebo. The participants will get the protection of Ad5-EBOV which can protect them from threat of Ebola virus in a period of time. At the same time, there may be some adverse reactions after injection. Common vaccination adverse reactions include: fever, tenderness and swelling on the injection site，redness. The adverse reactions are usually relieved in the 3-5 days after they occur.

It has been reported in other country's clinical study results that adenovirus vector may cause a prolonged clotting time in a period, but will not influence the safety of life generally. Also, adenovirus vector may increase the risk of HIV infection. But as the decrease of the level of neutralizing antibodies against adenovirus vector after vaccination, the risk of HIV infection will also decrease. So subjects will be closely followed to observe the infection rate of HIV for 6 months after vaccination. We will counsel subjects to avoid any behavior that will increase the risk of contracting HIV

In addition, the recent VSV vector vaccine clinical studies have found that vaccination may cause joint pain, which need to be observed in the study.

12.3.2. Vaccination

Regular qualified vaccination consumables will be made available together with sterile

inoculation following the standard method, strictly to avoid the adverse events caused by improper inoculation or mirrors.

If ≥grade3 serious adverse reactions or SAE that (maybe) related to the investigational vaccine occur during the safety observation period, the subjects should get immediate medical treatment. When necessary, Green channel for medical treatment should be started immediately for emergency treatment.

12.3.3. Blood Sample collection

Venous blood samples should be collected by experienced nurses who have gotten trained in accordance with the procedures after the qualification audit of the primary investigator to minimize the pain or danger of subjects（including pain and venous puncture site infection which is not common）

13. SIGNATURE PAGE OF SPONSOR'S APPROVAL FOR CLINICAL TRAIL PROTOCOL

Brief Title	A Phase II Clinical Trial to Evaluate the Recombinant Human Type 5 Adenovirus Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV)
Official Title	A Single-Center, Randomized, Blind, Phase II Clinical Trial to Evaluate the Safety and Immunogenicity of the Adenovirus Type 5 Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV) in Healthy Adults Aged Between 18 and 50 years in Sierra Leone
Vaccine	Recombinant Human Type 5 Adenovirus Vector Based Ebola Virus Disease Vaccine
Protocol Number	JSVCT024
Protocol Date	October 5, 2015
Version No.	1.3
Sponsor	Beijing Institute of Biotechnology, China Tianjin CanSino Biotechnology Inc., China
Sponsor Person In Charge	Wei Chen, PhD Beijing Institute of Biotechnology No. 20 Dongdajie street, Fengtai District, Beijing, People's Republic of China Zip code:100071 Tel:+8613910789661 Fax:+8610-63818253 E-mail: cw0226@foxmail.com
Sponsor Person In Charge (Sign) Signature Date	

STATEMENT OF PRINCIPAL INVESTIGATOR

I agree to:

- ✧ Take the full responsibilities as Principal Investigator of this clinical trial conducted by the institute.
- ✧ Ensure that this clinical trial is conducted according to this approved protocol, or revised protocol, and the clinical trial SOPs from Beijing Institute of Biotechnology and Tianjin CanSino Biotechnology Inc.
- ✧ Ensure that the investigators participating in this clinical trial understand the product information of investigational vaccine provided by the Sponsors, and understand the duties and responsibilities related to the clinical trial as outlined in this clinical trial protocol.
- ✧ Ensure that there are no changes to the clinical trial protocol without the review and written approval of the sponsors and the Institutional Review Board (IRB) unless it is due to urgent removal of immediate damages to the subjects or due to the regulatory requirements (such as due to administration requirements).
- ✧ I fully understand the correct usage methods of the investigational vaccine, and I fully understand the information provided by the sponsors, including but not limited to the following: Current Investigator Brochure or equivalent documents.
- ✧ I am familiar with and will comply with the requirements of Good Clinical Practices (GCP) and other relevant regulatory requirements.

Brief Title	A Phase II Clinical Trial to Evaluate the Recombinant Human Type 5 Adenovirus Vector Based Ebola Virus Disease Vaccine (Ad5-EBOV)
Protocol Number	JSVCT024
Protocol Date	October 5, 2015
Version No.	1.3
Principal Investigator	Dr Alie H Wurie National Ebola response, Emergency Operations Centre, Ministry of Health & Sanitation, Wilkinson Road, Freetown, Sierra Leone
Signature :	Signature Date

14. APPENDIX

Appendix 1 Recombinant Ebola Virus Vaccine (Ad5-EBOV) Phase II Clinical Trial Participant

Information Sheet (Version 1.2, Date October 5, 2015)

Appendix 2 Recombinant Ebola Virus Vaccine (Ad5-EBOV) Phase II Clinical Trial Participant Consent Form (Version 1.2, Date October 5, 2015)

MINISTRY OF HEALTH AND SANITATION
GOVERNMENT OF SIERRA LEONE

Recombinant Ebola Virus Vaccine (Ad5-EBOV) Phase II Clinical Trial
PARTICIPANT INFORMATION SHEET

1. General information about Ebola Virus Disease and the recombinant adenovirus type 5 vector based Ebola virus disease vaccine

Ebola Virus Disease (EVD) first appeared in 1976 in the southern region of Sudan and in the Democratic Republic of Congo near Ebola River, from which the disease takes its name. Five species of Ebola viruses have curently been identified. Among them, three Ebola viruses including Zaire Ebola virus, Sudan Ebola virus and Bundibugyo Ebola virus are mainly responsible for causing diseases in human, while Zaire Ebola virus is the most dangerous one. Drawing extensive concerns and attention in the world of medicine, Ebola virus is one of the most contagious and deadly viruses discovered so far. The virus is listed by the World Health Organization as Risk Group 4 Pathogen which means it is the most harmful to human. The disease can be quite severe and in the best centres only 3-5 out of 10 patients recover.

Currently many countries in the world are confronting with huge pressure on Ebola epidemic prevention and control, but so far there is no effective treatment for Ebola virus disease. The United States, Canada, Britain and other countries have been actively involved in the development and clinical evaluation of new Ebola vaccines. In the near future, vaccine will become an important measure for prevention and control of the Ebola disease.

In terms of the latest clinical research published on March 24, 2015, adenovirus type 5 vector based Ebola virus disease vaccine (Ad5-EBOV) has been developed by Chinese team, and it is safe and immunogenic with no serious adverse reactions based on its phase I clinical trial results in healthy Chinese adults and African adults living in China. 120 Chinese adults and 61 African adults living in China received this vaccine. Specific anti-Ebola humoral cellular immunities were detected in those participants.

2. What are we asking you?

We are asking you to participate in a Phase Ⅱ clinical trial of Ad5-EBOV. This is a single-centre, randomized and blind Phase Ⅱ clinical trial to evaluate the safety and immunogenicity of Ad5-EBOV in healthy adults in Sierra Leone and to determine the appropriate immune dose.

3. What will you be asked to do if you decide to receive this vaccination?

You will be randomly assigned to receive high dose vaccine (1.6×10^{11}vp/vial), or low dose vaccine (8×10^{10}vp/vial), or placebo according to a ratio of 2:1:1. You have 50% possibility to be assigned to receive high dose vaccine, 25% possibility to receive low dose vaccine and 25% possibility to receive placebo.

- You will be given two shots of vaccine intramuscularly in upper arms, one injection on each arm.
- You will be observed for at least 60 minutes after receipt of vaccination in the study centre for occurrence of any adverse reactions.
- You will be followed for adverse reactions symptoms, health condition, medical treatment, medicine and other vaccine used within the first 7 days after vaccination.
- You will be asked to donate 8ml blood at the first visit (Day 0), and 5 ml blood sample at the later visits (Day 14, Day 28 and Month 6). A total of 23 ml blood you need to donate for immunogenic tests throughout the study.
- This study lasts for 6 months. You need to return to the study centre on Day 14, Day 28 and Month 6 post-vaccination.

4. Can you change your mind after saying “Yes”?

Yes, you can change your mind at any time. If you wish to withdraw, just tell your doctor. Your decision will not stop you from getting usual medical care.

5. What is the benefit from receiving this vaccination?

We cannot promise that the vaccination of Ad5-EBOV may elicit antibodies against Ebola virus, and the vaccine cannot guarantee that you will not suffer from Ebola virus disease. However we believe that your contribution to this study will be important to the development of this recombinant Ebola virus disease vaccine and might benefit other people to fight against the disease in the future.

6. **What will you receive for taking part in the study?**

We will pay you 10 dollars (about 50000 SLL) as transportation fee and overtime meal allowance at each clinic visit. You will receive a cell phone for the safety follow-up after vaccination.

7. What are the risks of receiving this vaccination?

Risks of injection: Some adverse reactions like the erythema, induration, swelling, itching and so on in the inoculation positions are generally very light, they will be relieved or disappear, and don't need special treatment. If necessary, ask doctors for symptomatic treatment timely. The possibility of infection is very low.
Risks of blood sampling: Blood sampling sites may have erythema and mild pain. Although syncope during blood sample selection, or sampling sites infection are very rare, these may also occur sometimes.
Risks of allergy: Rapid onset of severe allergic reaction after the vaccination is very rare, but could be potentially life-threatening. So medical staff will observe and evaluate your health status during your stay in the clinic centre after vaccination. They will give you proper treatment immediately if allergic reaction occurs.
Risks of investigational vaccine: Based on the results of Phase I clinical trial in healthy Chinese population and other animal studies, we believe that the safety of the vaccine is acceptable. We will closely observe the adverse reactions after vaccination. According to data of clinical studies and reports of similar vaccines conducted by other countries, this vaccine may cause fever, injection site pain, arthralgia and other adverse reactions. HIV vaccine clinical trials in other country have observed that adenovirus type 5 vector HIV vaccines may increase the risk of HIV infection of subjects. Although there is no evidence that other adenovirus type 5 vector vaccines also increase the risk of HIV infection, we do not exclude the possibility of such a risk. So you need to pay attention to avoid any dangerous behaviors which can cause HIV infection during the whole study period, such as unsafe blood transfusion, unsafe sex life and drug taking, etc.

8. **What if something goes wrong with you?**

If you have been infected by HIV or a serious adverse event (SAE) occurs during the study, please report to the study physician (**Dr. Deen, Tel. 078801733**) immediately. You will get appropriate medical care in time. According to your health conditions and the items in your insurance bought by the sponsor, you may get financial compensations.

9. Do you have other choices?

You can choose to participate in this trial or not. Your choice will not affect the usual medical care. We will always do our best to take care of you. If you agree to participate, you will also be helping us learn whether the vaccine works and how it works to help other people, though you can withdraw at any time.

Currently, there is no effective treatment or promising drugs approved for Ebola virus disease. In terms of global market, there are several candidate vaccines in the development stage but there are no approved commercial products launched. If the risk of Ebola virus infection increases, one can enhance personal protections, including avoiding contact with the patient or the patient's body fluids, secretions and contaminants to prevent infection.

10. What cost will be paid?

You will not have to pay anything to participate in this study.

11. How will your privacy be protected?

All research data as well as your identity and personal information will be kept confidential and will not be open to the public. We will assign you a code to identify you in the study, your name and other information will be kept strictly confidential. Your name will not appear in any published information or reports of the study. We will make every effort in the extent permitted by law to protect the privacy of your personal medical information. The test results during the study will be used only for this analysis, and will not be used for any other research, and will not be disclosed to others. Your personal information will be hidden when submitting to the sponsor (Beijing Institute of Biotechnology and Tianjin CanSino Biotechnology Inc.). But the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject.

12. Who would you talk to?

If you have questions, concerns about the vaccine or medical problems or would like more information or further clarification, you can talk to: **Dr. Alie H Wurie, Tel:076801478 and Dr James B W Russell, Tel: 078801266**

If you have any questions about your rights or welfare as a research subject, please contact **Prof. Hector Morgan, Tel: +23276629251**, Director of the Research Ethics Committee, Freetown, Sierra Leone.

Your signature documents your permission to use this experimental vaccine.

Participant's Name: ……
Please Print

Signature/thumb print: …… **Date**:……. **Time**: …….. …
DD MTH YEAR
00H00

- **If the person giving the consent cannot read the form by themselves, a witness must be present and sign here.**

  I have witnessed the process of reading the consent form to this patient. The form was read appropriately and questions raised by the participant were addressed adequately. I hereby confirm that the participant gave consent without undue influence or coercion.

Witness Name: ……..
Please Print

Signature: …… **Date**:……. **Time**: …….. …
DD MTH YEAR
00H00

I, the undersigned physician have fully explained the relevant information of this trial to the person named above and will provide her/him with a copy of this signed and dated informed consent form.

Name of Physician: ……
Please Print

Signature/thumb print: …… **Date**:……. **Time**: …….. …
DD MTH YEAR
00H00

MINISTRY OF HEALTH AND SANITATION
GOVERNMENT OF SIERRA LEONE

Recombinant Ebola Virus Vaccine (Ad5-EBOV) Phase II Clinical Trial

PARTICIPANT CONSENT FORM

I understand that the main aim of the study is to determine the safety and immunogenicity of one vaccination of Ad5-EBOV in healthy adults in Sierra Leone and to explore the appropriate immune dose. The information will help to evaluate the vaccine, and hopefully, to fight against the epidemic of the Ebola virus and to protect people from suffering Ebola Viral Disease.

I understand that I will be required to give information about my personal information. I will also undergo a physical examination and receive investigative vaccines. I will donate 8 ml blood samples at the first visit (Day 0) and 5 ml blood sample at the later visits after vaccination (Day 14, Day 28 and Month 6). A total of 23 ml blood I need to donate for immunogenic tests throughout the study. The study will last for 6 months with 4 clinic visits (Day 0, Day 14, Day 28 and Month 6).

I will be observed for at least 60 minutes in the study centre after vaccination and will be followed by an assigned staff within the first 7 days after vaccination for safety observation. I understand that my participation in this study is entirely voluntary and that I am free to withdraw from the study at any time without affecting my future health care.

All the information about me will be kept strictly confidential and used only for the trial. If at any time I have questions relating to the study I am free to contact the principal investigator: **Dr. Alie Wurie Tel.: 076801478**

I voluntarily agree to take part in the phase Ⅱ clinical trial of adenovirus type 5 vector based Ebola virus disease vaccine (Ad5-EBOV). I am willing to take the investigational vaccine as prescribed and I have been offered a copy of this information form.

Participant's Name: ……
Please Print

Signature/thumb print: …… **Date**:…….. **Time**: …….. …
DD MTH YEAR
00H00

- **If the person giving the consent cannot read the form by themselves, a witness must be present and sign here.**

  I have witnessed the process of reading the consent form to this patient. The form was read appropriately and questions raised by the participant were addressed adequately. I hereby confirm that the participant gave consent without undue influence or coercion.

Witness Name: ……..
Please Print

Signature: …… **Date**:…….. **Time**: …….. …
DD MTH YEAR
00H00

I, the undersigned physician have fully explained the relevant information of this trial to the person named above

and will provide her/him with a copy of this signed and dated informed consent form.

Name of Physician: ……..……

Please Print

Signature/thumb print: …… **Date**:……. **Time**: …….. …

DD MTH YEAR

00H00