

Progress of vaccine and drug development for Ebola preparedness

Clin Exp Vaccine Res 2015;4:11-16
<http://dx.doi.org/10.7774/cevr.2015.4.1.11>
 pISSN 2287-3651 • eISSN 2287-366X

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Received: December 22, 2014
 Revised: December 29, 2014
 Accepted: December 30, 2014

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No potential conflict of interest relevant to this article was reported.

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Since the first case of Ebola virus disease (EVD) in Guinea was reported in March 2014 by World Health Organization (WHO), the outbreak has continued through the year and the total number of 19,065 patients was reported as the confirmed or suspected in the EVD-affected countries. Among the cases, 7,388 patients were reported death by 19 December. Currently, available therapeutics to treat the infected patients or vaccines to prevent people from infection is not developed yet while viral diagnostic methods were already developed and firmly established in a lot of countries as a first step for the preparedness of Ebola outbreak. Some potential therapeutic materials including ZMapp were supplied and the treated people got over the EVD. Several candidates of vaccines also were investigated their efficacy in animal models by National Institute of Health (NIH) and Department of Defense, and they are processing of clinical tests in West Africa aiming to finish the development by the 2015. Vaccine and therapeutic development is essential to stop the EVD outbreak in West Africa, also to protect the world from the risk which can be generated by potential spread of Ebola virus.

Keywords: Ebola virus, Vaccines, Drug development, Ebola hemorrhagic fever

Introduction

During nine months till the middle of December in 2014, more than 19,000 people were infected by Ebola virus (Fig. 1) and lots of the patients around 40% were dead by the Ebola virus disease (EVD). The outbreak through the year seems to keep increasing the number of cases and deaths unless efficient drugs or vaccines would not be supplied. Also whole world are nervously focusing on the upcoming situation in the Ebola virus-affected countries in West Africa day by day. Since the first case of EVD was reported in March 2014, the outbreak has continued and the total number of 19,065 patients was reported as the confirmed or suspected in the EVD-affected countries, mostly happened in the three outbreak countries (Guinea, Liberia, and Sierra Leone) [1]. United States, Spain, Mali, and two other previously affected West African countries (Nigeria and Senegal) also have reported a few cases. Among the cases, 7,388 patients were reported death (as of December 19, 2014).

EVD is an acute pathogenic symptom caused by expose to the Ebola virus, one of four major hemorrhagic fever viruses (HFVs) generating harsh illness. The RNA viruses of these families causing hemorrhagic fever include *Flaviviridae*, *Arenaviridae*, *Bu-*

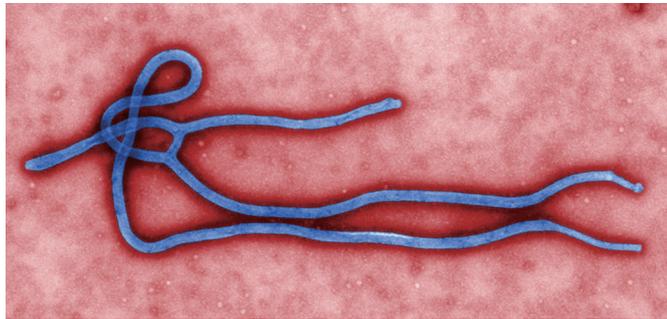


Fig. 1. Colorized transmission electron micrograph of Ebola virion (courtesy to Centers for Disease Control and Prevention).

nyaviridae, and *Filoviridae* [2]; yellow fever virus and Dengue virus are included in *Flaviviridae*, and they are usually transmitted by mosquito as a vector. Lassa virus and several South American HFVs transmitted by small rodent contact are well known pathogens of *Arenaviridae*, and critical members of *Bunyaviridae* may cause the Rift Valley fever (RVF), Crimean-Congo hemorrhagic fever (CCHF), and Hantaan virus is also a member of the family. RVF virus is transmitted by mosquito while CCHF virus is transmitted by ticks. The most notorious HFVs are Ebola virus and Marburg virus which are classified as members of family *Filoviridae*, first Ebola virus was found in the area around Ebola River in Congo in 1976, and Marburg virus was discovered in the German city, Marburg in 1967. However, the ecology and the epidemiology of Ebola virus are not clearly understood or natural host is not confirmed either even though they are considered one of the most highly dangerous pathogens and potential threatening to the humankind. The viral isolates from the patients in Guinea this year have high homology (98%) with Zaire strain of Ebola virus (ZEBOV) isolated from the patients of Congo and Gabon during 1994-1995 [3]. ZEBOV is the most lethal pathogen among five reported Ebola viral strains, and it was known to cause over the 90% of fatality in human and primates [4]. This virus may cause acute symptoms in infected patients from 2 to 21 days after exposed to the virus; high fever, bleeding, disseminated intravascular coagulation, headache, abdominal pain, non-bloody diarrhea, myalgia, nausea, arthralgia, and malaise [5].

As the diagnostic methods for Ebola virus, enzyme-linked immunosorbent assay (ELISA) and real-time reverse transcription-polymerase chain reaction (rRT-PCR) are generally used. While antigen or antibody (IgM/IgG) can be detected by ELISA, rRT-PCR is the most common and useful diagnosis technique by which the existence of Ebola virus-specific genes

can be investigated after virus isolation. Among these diagnostic methods, antibody-capture ELISA is limited to be applied for the early diagnosis because antibodies usually do not appear within 1 or 2 week of illness [6]. Virus isolation is also limited to apply for any situation because it requires a biosafety level 4 laboratories to perform this step. After establishment of diagnostic methods as the first step for the preparedness against EVD, therapeutic materials should be acquired to rescue the infected patients and suitable form of vaccine may be required to prevent the population from infection. However, the development of therapeutics and vaccine for EVD is still at the early stage and the proceeding is very slow because the most procedure during the research and development related to Ebola virus is always highly risky and requires special protective facilities such as biosafety level 4 laboratories. Many scientists and companies cannot perform the research related to selected agents such as Ebola virus due to this limit of risk and facility even though they have willing to contribute to the progress of drug and vaccine development.

Ebola outbreak in West Africa this year asked world to reveal the potential to protect people from the dangerous pathogen, and now we realize there are some positively expected candidates of therapeutics and vaccine. Some of the therapeutics was already used for the treatment of patients in the United States and Europe, and several vaccine candidates showing efficacy in animal tests were on the clinical tests in West Africa now.

Drug Development for the EVD Treatment

Drug development for the EVD treatment started around 2002 just after 911 in United States, and it was continuously supported by governmental institutes such as National Institute of Health (NIH), Biomedical Advanced Research and Development Authority and Defense Threat Reduction agency of United States, also Public Health Agency in Canada. Based on the financial and technical support from governments, United States and Canadian companies have tried to develop some available forms of anti-Ebola therapeutics (Table 1).

First of all, well-known antibody therapeutics named ZMapp was under development led by Leaf Biopharmaceutical Inc. (San Diego, CA, USA) from 2004. ZMapp is an antibody cocktail mixing the humanized mAbs with the selected composition of c13C6 from MB-003 (human-mouse chimeric mAbs developed by Mapp Biopharmaceutical Inc. located in San

Table 1. Drug and vaccine candidates for the clinical trials

Candidate	Manufacturer		Format	Clinical trial
Drugs	ZMapp	Mapp Biopharmaceutical, USA	Plant-derived antibody cocktail	Not yet
	TKM-Ebola	Tekmira, Canada	RNAi	Phase I
	Favipiravir	Toyama Pharmaceutical Co., Japan	RNA polymerase inhibitor	Phase I
Vaccines	cAd3	GSK, UK (developed by NIH, USA)	Chimpanzee adenovirus-based DNA vaccine	Phase I
	VSVΔG-ZEBOV	NewLink Genetics Co., Canada (developed by Public Health Agency of Canada)	Recombinant vesicular stomatitis virus-based VLP vaccine	Phase I

VLP, virus-like particle.

Diego, CA, USA) and c2G4 and c4G7 from ZMAb (mouse mAbs developed by DeFyus located in Toronto, Canada) [7,8]. Both MB-003 and ZMAb are also cocktails of Abs; MB-003 is a mixture of human and human mouse chimeric mAbs (c13C6, h13F6, and c6D8), and ZMAb contains three mouse mAbs (m1H3, m2G4, and m4G7). The components of ZMapp was produced in the tobacco plant (*Nicotiana benthamiana*) at the farmyards using biopharming technology carried out by the Kentucky BioProcessing after development funded by Defense Advanced Research Projects Agency. ZMapp was designed and produced through two major steps: first of all, the mouse was inoculated with Ebola virus, then the immunological memory on the mouse lymphocytes by the infection was cloned. As a second part, cloned genes containing the memory related to Ebola virus was transferred to the tobacco plants by transformation using bacterial carrier. Finally plants expressing anti-Ebola mAbs were cultured in the fields and the antibodies were purified after harvest. Produced ZMapp showed enough efficacies to protect chimpanzees from Ebola virus, however it was not tested yet before this outbreak in West Africa. ZMapp was supplied and helped to rescue the first American Ebola-infected patients this summer; however, another patient in Spain was not recovered even the ZMapp was treated. Even it seems efficient material to rescue patients, ZMapp still has critical limitation to be released to the clinical fields as a common Ebola therapeutics due to its slow procedure of production.

In addition to ZMapp, there are also several other well-recognized EVD therapeutic candidates including TKM-Ebola and favipiravir. TKM-Ebola developed by Canadian company Tekmira is a drug using RNAi form designed to block the replication of the Ebola virus. Though it showed a good efficacy in animal tests and also applied to clinical phase II, the potential safety issues may be a limit to be quickly supplied to the clinical fields. However, the TKM-Ebola can be a one of important candidates to be applied for current West African

outbreak because large volume of the RNAi can be produced within comparatively short period and another product TKM-Marburg also showed highly efficient protection in chimpanzee tests from infection of Marburg virus. As small molecules which can be easily synthesized by chemical reaction, favipiravir is considered as a top candidate to be produced for West Africa. Favipiravir, also called as Abigan of commercial name, is an RNA polymerase inhibitor developed by Japanese company Toyama Chemicals (a subsidiary of Fuji Film) and already under the clinical phase III as a new influenza treatment. This new drug was quickly focused as an efficient Ebola hemorrhagic fever (EHF) treatment at the early stage of the outbreak in West Africa because the role of RNA polymerase also has the most important role during the replication of Ebola virus similar to the replication of influenza viruses. Now favipiravir is under the test performed at the NIH, and Japanese company is ready to send the inhibitors to West Africa if the efficacy for EVD treatment would be confirmed and Japanese government would approve to use. In addition to the candidates, there is several reports that other antiviral drugs such lamivudine which is an anti-human immunodeficiency virus (HIV) drug may show efficacy to help the EVD patients.

Quick development of efficient drugs to be treated is the most urgent issue to rescue a lot of Ebola-infected patients in West Africa, and World Health Organization (WHO) is considering supplying some therapeutic candidates such as TKM-Ebola or favipiravir to the Ebola-affected countries in Africa sooner or later to suppress the situation till the production of an effective vaccine.

Vaccine Development for the EVD Prevention

Because the Ebola virus did not frequently generate outbreak, vaccine research and development was not eagerly preceded. Vaccine candidates were just tested at the preclinical levels using animals, and the development of manufacturing pro-

cess was unpredictable step for the most potentially harmful pathogens such as HFVs including Ebola virus. However, the vaccine research has been continued for long history after virus identification in 1976 and some candidates were already confirmed about the efficacy in chimpanzees [9,10]. Similar to the other vaccines, live attenuated vaccines was considered and studied as a first candidate; efficacy and safety were tested using nonhuman primates as primary animal models. However, the results did not discover any effective vaccine candidates even in the level of animal model. Even though there was Ebola vaccine studies continued for last decade, the progress was not so fast or productive because the most procedure for the development of vaccines related to selected agents such as Ebola virus has many limitations about the safety facilities and personal expertise. It is very difficult for the private research groups or companies to handle the selected agents or maintain the high safety level facilities, and usually the most steps of research were performed by limited agencies. Recently, some vaccine approaches using DNA vaccine type containing Ebola antigen-coding genes announced positive results in animals. Vaccine research center at NIH and developed an efficient candidate with adenovirus vector, and British research group at Cambridge University studied another candidate for the veterinary vaccine in variable animal models to protect primates from Ebola virus in Africa while early animal study of live attenuated vaccine with Guinea pigs were not satisfied to be effective form of vaccine.

DNA vaccine as Ebola virus vaccine candidate was tried during early 2000s and several positive results were suggested as possible direction. In DNA vaccine type for EVD prevention, inserted nucleoprotein or glycoprotein (GP) of Ebola virus acts as an antigen to induce host immune responses and inserted antigen-delivering DNA may boosts the immunization [11]. The DNA vaccine candidate was continuously studied using animal models such as guinea pigs or chimpanzees with results of high protection efficacy [12,13]. However the efficacy in primates including human was not as high as the level of rodents [14] though the plasmid containing GP maintained protective memory for long time in animals when Ebola virus was challenged [15]. Therefore many research groups including NIH Vaccine Research Center tried to develop priming protocol to improve efficacy, and now there are two selected protocols based on the combination of viral vectors to remarkably enhance the immune responses in human and nonhuman primates (Table 1) [16,17].

cAd3 Ebola Vaccine

After long time efforts related to develop vaccine platform using recombinant chimpanzee adenovirus vector for Ebola vaccine, Okairos (acquired by GSK) manufactured cAd3 Ebola vaccine (recombinant chimpanzee adenovirus serotype 3 vectored Ebola vaccine) and this candidate has been tested for clinical phase I by GSK.

Adenovirus vector has been considered as an effective platform for the DNA vaccine for a wide range of infectious pathogens such as HIV and tuberculosis, and the vaccine research center of NIH studied availability of cAd3 Ebola vaccine containing Ebola GP gene to be expressed in hosts after vaccination. Chimpanzee adenovirus vector seems to be safe because the animal vector do not replicate in human hosts and the cAd3 already showed hopeful result that protected all 16 animals from EHF after single vaccination [11,18]. There are two adenovirus vector-based Ebola vaccine candidates; monovalent vaccine against only Zaire strain, and bivalent vaccine against Zaire strain and Sudan strain. The monovalent vaccine is the candidate scheduled on September 2014 for the phase I clinical test in West Africa. Safety and immunogenicity of the cAd3 in human hosts will be announced in 2015.

Recombinant Vesicular Stomatitis Virus Ebola Vaccine

Recently, trial using vesicular stomatitis virus (VSV) platform designed as bivalent vaccine against Ebola and Marburg viruses revealed systemic immune responses protecting animals after injection [19]. This non-segmented, negative stranded RNA virus is also considered as a promising candidate for the recombinant DNA vaccine platform against many filoviruses because the virus is an animal pathogen which usually does not induce any severe symptoms in human [20, 21], and the most advanced form using VSV for Ebola vaccine is VSVΔG-ZEBOV vaccine developed and sponsored by the Public Health Agency of Canada and NewLink Genetics Corporation. The VSVΔG-ZEBOV vaccine contains highly attenuated recombinant VSV with substituted Ebola virus Zaire envelope GP and it can be cultured quickly for high titer. The VSVΔG-ZEBOV vaccine candidate also revealed the 100% protection efficacy in animals, furthermore this form of vaccine was administered for a postexposure human patient injured by laboratory accident in German research group. This candidate is also to be conducted in African countries.

Conclusion

NIH keeps the vaccine research for 10 years using various formats and announced clinical test in West Africa based on these results last September. WHO and whole world may expect successful process by the 2015 for the Ebola vaccine development. The first clinical trial in Ebola-affected area was scheduled in October 2014 and the results will be shown sooner or later.

Even though the EVD is still in outbreak and keeps spreading in Africa, there are no effective vaccines to protect people or no approved therapeutics to rescue the infected patients either. It seems long way to stop the current outbreak in Africa, also to clearly extinguish the threatening generated by the highly fatal pathogens such as Ebola virus. However, saving stockpile of vaccines after quick process of development will be the most effective way to prepare the crises related to the biological agents. As a warning of EVD outbreak which has lethal fatality just after infection, we have to recognize the request for vaccine development with huge scope of potential pathogens which can be threatening in the future. Vaccination should be ultimate responsiveness against outbreak while the efficient therapeutics is urgently requested for the treatment of patients in the affected countries. It is the best strategy to quickly make current vaccine development successful to prevent the world from spread of Ebola virus.

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