Activating Antibody Warriors to Fight the *Ebola Virus!*

Photo Credit: National Institute of Allergy and Infectious Diseases's

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Transformation of Original Research Article

Pascal, K.E., Dudgeon, D., Trefry, J.C., Anantpadma, M., Sakurai, Y., Murin, C. D., Turner, H. L., Fairhurst, J., Torres, M., Rafique, A., Yan, Y., Badithe, A., Yu, K., Potocky, T., Bixler, S. L., Chance, T. B., Pratt, W. D., Rossi, F. D., Shamblin, J. D., … Kyratsous, C. A. (2018). Development of clinical-stage human monoclonal antibodies that treat advanced Ebola virus disease in nonhuman primates. *Journal of Infectious Diseases*, 218(5), S612-S626. https://doi.org/10.1093/infdis/jiy285

Abstract

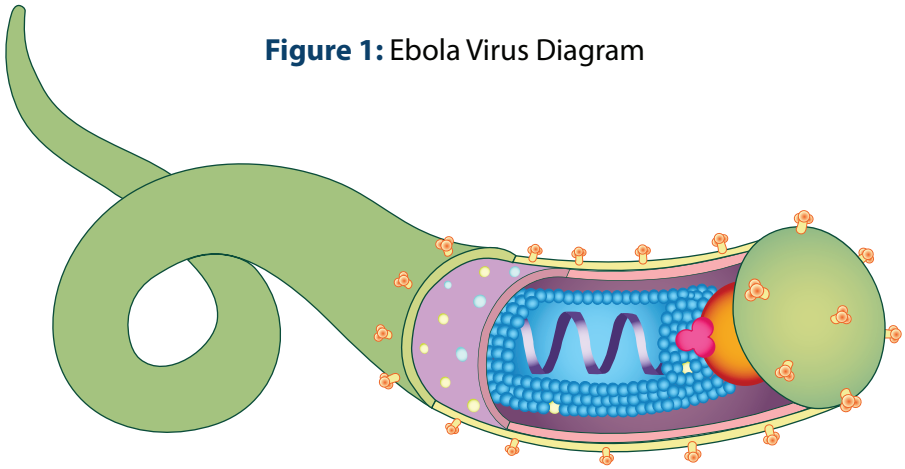
Ebola virus disease (EBOV), commonly known as Ebola, is a severe and often fatal illness in humans. There are currently no approved therapies or vaccines for the Ebola virus. Instead, treatment for Ebola is limited to supportive care such as fluids, electrolytes, and pain management. Scientists investigated ways to boost the body's own immune system to fight Ebola. Antibodies found in blood samples of survivors of EBOV were used to develop an effective therapeutic. Three monoclonal antibodies (derived from the same source) were tested at different pH levels to identify epitopes (bonding sites) on the EBOV. A therapeutic mixture of these antibodies were given at different dosages to determine treatment efficacy. The epitope interactions with the three different monoclonal antibodies resulted in a significant decrease in viral RNA. Data from this study provided evidence to move forward with clinical trials to treat EBOV.

Background

During a three-year period, from 2013 through 2016, over 28,000 people were infected with *Zaire ebolavirus* (**EBOV**). Of those infected, 11,000 people died. For those who survived, they continue to suffer with lasting health issues caused by the virus. These include ***neurological*** and ***ocular*** (vision) complications, joint and muscle pain. Some survivors unknowingly serve as a host for persistent EBOV infection. Persistent EBOV infection means the Ebola virus can "hide" in the body, specifically in the brain. In men, EBOV can also hide in testicles. These hidden viruses can re-emerge, causing the individual to be reinfected with EBOV. Studies have shown the reinfection occurs about 15 months after the initial infection.

Currently, there are no approved therapies or vaccines for EBOV, but infectious disease researchers are working to change that. They are turning their attention to the body's own immune system to fight EBOV. When a foreign body, like EBOV, enters the body, the immune system recognizes an invader. The invader is called an antigen (AN-tah-gen). Antigens can be bacteria, fungi, or viruses like Ebola. The immune system creates antibodies to attack the antigen. Antibodies target specific structures on the surface of the invading antigen and kill it or surround the antigen to prevent its spread.

Figure 1: Ebola Virus Diagram



The Ebola virus is not alive. However, it does contain genetic material: single-stranded ribonucleic acid (RNA). When an Ebola virus attaches to the surface of a living cell, it enters the cell where it releases its RNA strand into the cell. Once in the cell, the virus uses the cell's organelles to read the virual RNA strand and replicate. As the EBOV replicates, the cell becomes filled with EBOV until it bursts, releasing even more EBOV into the body.

Not everyone who is infected with EBOV dies. With permission from survivors, infectious disease researchers collected blood samples and tested these samples, looking for clues as to how these people survived the infection. The researchers found antibodies that targeted a specific structure on the surface of EBOV. This structure is a glycoprotein (gly-co-PRO-teen). Through their tests on the survivors' blood, researchers found antibodies bonded to the glycoprotein (GP) on the surface of the EBOV. They determined these antibodies prevented the EBOV from entering the body's cells. The EBOV needs to get inside cells to replicate. If it cannot get into the cell, the virus cannot replicate, preventing EBOV disease.

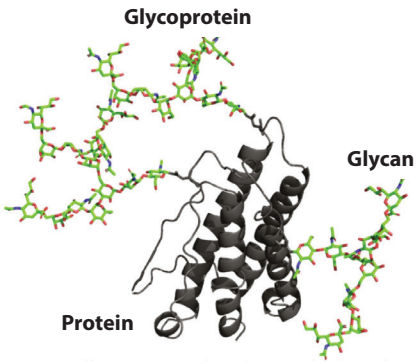
In this study, scientists developed a platform to generate, select, test, and manufacture fully human therapeutic antibodies to treat EBOV. To ensure therapeutics would work for humans, this study used only human antibodies obtained from EBOV survivors and rodents genetically engineered to produce human antibodies.

Methods

Before scientists could search for therapeutics, they had to figure out how to safely work with EBOV. Working collaboratively with multiple universities and research centers, scientists developed an Ebola ***pseudovirus***. The surface of the pseudovirus has the same GPs as EBOV but there is nothing inside the pseudovirus. Using pseudoviruses, scientists could safely test antibody/GP interactions without risk of contracting EBOV. Their tests sought to identify specific antibodies as possible therapeutics to prevent EBOV from injecting its RNA into human cells where it can replicate, causing death, widespread illness or even a pandemic.

To develop an effective therapeutic, scientists set out to identify antibodies which were effective at bonding with GPs on EBOV. Building on prior research, the scientists understood an effective therapeutic would not be developed from a single antibody but would need to be a combination of different antibodies.

Glycoproteins on the surface of EBOV are complex structures. As the name implies, GPs are made of two types of structures: glycan and protein. Glycan is a sugar molecule. The protein is made of amino acids, like those found in DNA and RNA.



Source: ScienceDirect

In Vitro Methods

In vitro methods means scientists conducted their experiments on non-living organisms: think petri dishes and test tubes. From the range of GP-specific antibodies tested, scientists looked for data-supported outcomes which tested antibodies' ability to: 1) block the GP on the pseudovirus EBOV from entering cells to replicate, 2) remain attached to the GP at different pH levels which would prevent the antibodies from interacting with other surface receptors, 3) not be in cross-competition with other antibodies, 4) bind to GPs to block potential EBOV pathogenesis, and 5) mediate killing of EBOV-infected cells by immune cells. Of the antibodies tested, three antibodies best fit these requirements, known as ***metrics***. The antibodies are referred to as REGN3470, REGN3471, and REGN3479.

Once the most promising therapeutic antibodies were identified, the scientists needed to generate more of these antibodies. For purity, each of the three antibodies were cloned from a single source within the lab. Because the antibodies were cloned from this single source, they are called monoclonal ***antibodies*** (mAbs). After cloning, the three mAbs were combined into a therapeutic mixture ready for testing on wild type EBOV.

But there are challenges to creating a sufficient supply of effective therapeutic antibodies for EBOV. Afterall, Ebola is a deadly disease. Up to this point, scientists used pseudoviruses to test antibodies using in vitro methods. The use of pseudoviruses permitted the scientists to safely conduct the in vitro experiments. However, to truly test the effectiveness of the therapeutic mixture, scientists needed to test it on active EBOV, known as wild type EBOV in living organisms, meaning shifting from in vitro to in vivo studies.

In Vivo Methods

This phase of the research switched from conducting in vitro experiments using pseudovirus to conducting in vivo methods studying the effectiveness of the therapeutic antibodies on wild type EBOV in living organisms. To work with this deadly virus, the study needed to move to a lab specifically designed for working with deadly pathogens.

Honeywell North© BSL 4 Air-Fed Suit

Labs are categorized by number. The higher the number, the more personal protective equipment (PPE) a scientist needs to wear. The highest rated lab is a biosafety level 4 lab (BSL4). In a BSL4 lab, scientists work with pathogens which have no cure or proven treatment, like EBOV. To keep scientists safe, they wear full-body positive pressure suits which is hooked up to an air supply. Using maximum safety protocols, scientists could now work with wild type Ebola using in vivo methods.

In vivo studies involve testing therapeutics on living organisms, in this case rodents and nonhuman primates (NHP) were used. The efficacy of each of the three potential therapeutic antibodies were tested separately on rodents infected with EBOV. Scientists evaluated the effectiveness of each antibody at treating rodents challenged with EBOV. Blood samples from the rodents were tested for antibody levels while their overall health was monitored for EBOV symptoms.

Photograph by Constance Dubuc

The next phase of the study transitioned to an NHP model, specifically Rhesus macques. The physiology of NHPs closely aligns with that of humans. To better assess the efficacy of the mAbs mixture, NHPs infected with EBOV were treated with mAbs. As with the rodents, blood samples were taken and tested for levels of mAbs and EBOV while monitoring the NHPs for EBOV symptoms.

Results

Building on previous research, this study sought to develop an effective mixture of noncompeting EBOV GP specific, fully human antibodies that use different mechanisms to combat EBOV. The resulting 3-antibody mixture possess diverse biological mechanisms of action which compliment one another. The in vivo studies with NHPs provided data evidence as to the effectiveness of the 3-antibody mAbs mixture to treat EBOV.

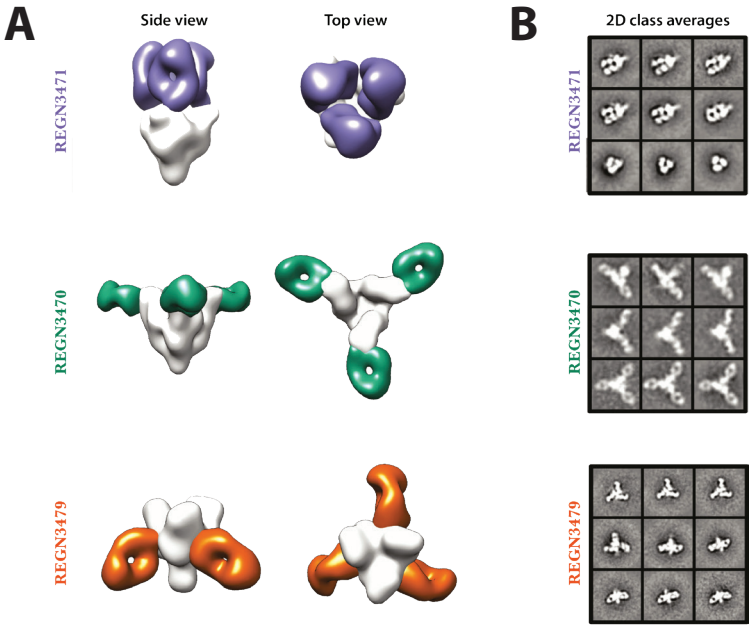
All three antibodies demonstrated high affinity, remaining bonded to the GP at different pH values (Table 1). The natural pH of blood is 7.4; however, pH values may change as the EBOV disease progresses. In addition to showing bonding stability at different pH values, each of the antibodies did not compete for bonding sites on the GPs. These bonding sites are called ***epitopes (EP-ah-topes)***. Each antibody binds to a separate epitope, meaning there is a unique attachment site for each of the three antibodies on the GP (Figure 2).

Table 1. Dissociation Kinetics of REGN3470, RENG3471, and REGN3479 from EBOV at pH 7.4, pH 6.0, and pH 5.0

Antibody	k_d (1/s)	$t_{1/2}$ (min)	k_d (1/s)	$t_{1/2}$ (min)	k_d (1/s)	$t_{1/2}$ (min)
REGN3470	4.32×10^{-4}	26.7	3.67×10^{-4}	31.4	4.91×10^{-4}	23.5
REGN3471	1.95×10^{-4}	59.1	2.21×10^{-4}	52.3	3.32×10^{-4}	34.8
REGN3479	7.83×10^{-5}	147.6	2.14×10^{-4}	55.1	2.02×10^{-4}	57.3

Abbreviations: EBOV, Ebola virus; GP, glycoprotein.

Figure 2: (A) Side view and top view of epitopes for each antibody on the GP; (B) Electron microscope copy of REGN3470, REGN3471, and REGN3479 bound to EBOV GP.

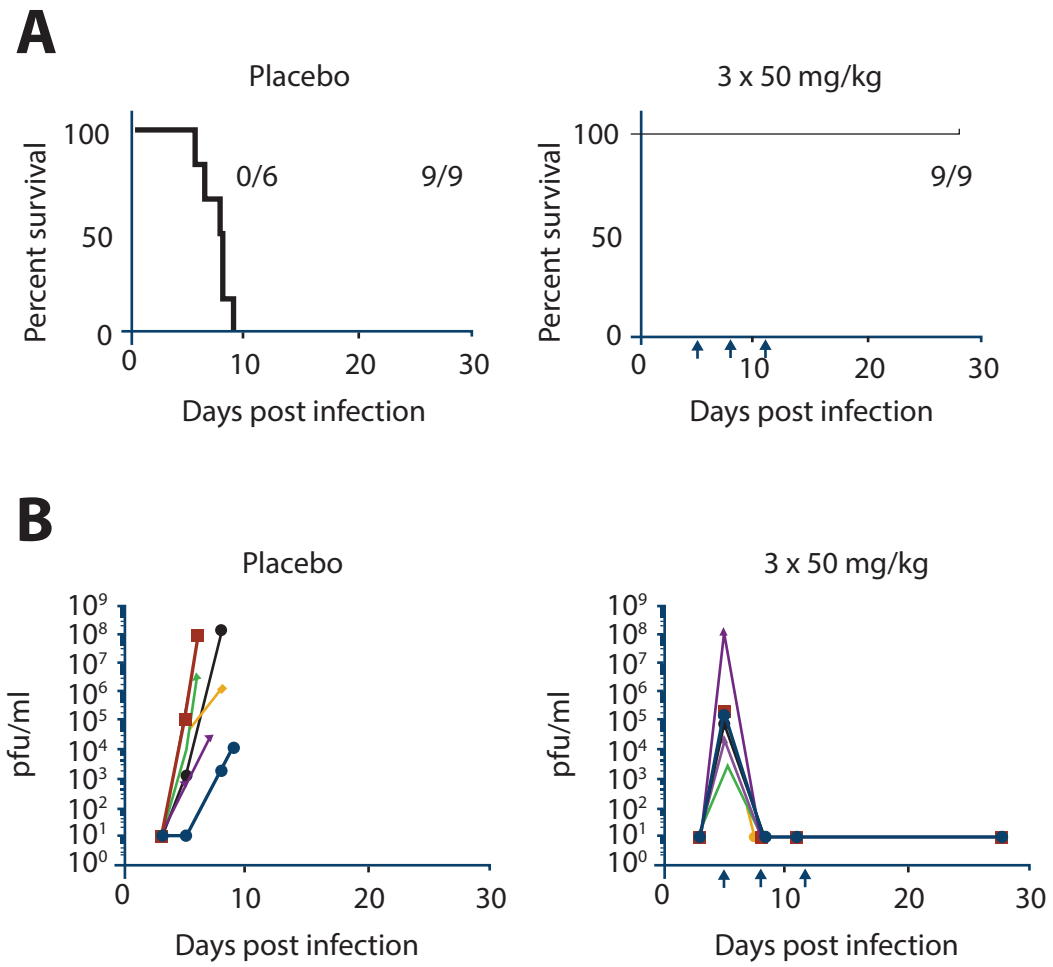


Having separate epitopes indicate the antibodies are noncompeting for attachment sites on GPs. Importantly, each of the three antibodies bind simultaneously to EBOV GPs. All three antibodies used different, yet complimentary mechanisms to neutralize EBOV. Outcomes from both in vitro and in vivo studies demonstrates the 3 mAbs provide protection from EBOV.

Therapeutic Efficacy

Therapeutics must be thoroughly tested for effectiveness and safety. Using a control/intervention approach, scientists observed NHPs infected with EBOV. From observations in the field, scientists knew the disease reaches a critical point on day 5 with a spike in temperature. After day 5, individuals infected with EBOV exhibit increased symptoms and generally expire by day 10. At day 5, infected NHPs from the intervention group were given the 3 mAbs therapeutic. They responded with decreased temperature and a reduction in symptoms. The 3 mAbs provided the NHPs with 100% protection from the lethal disease (Figure 3).

Figure 3: Testing of 3 mAbs Control/Intervention Testing Outcomes

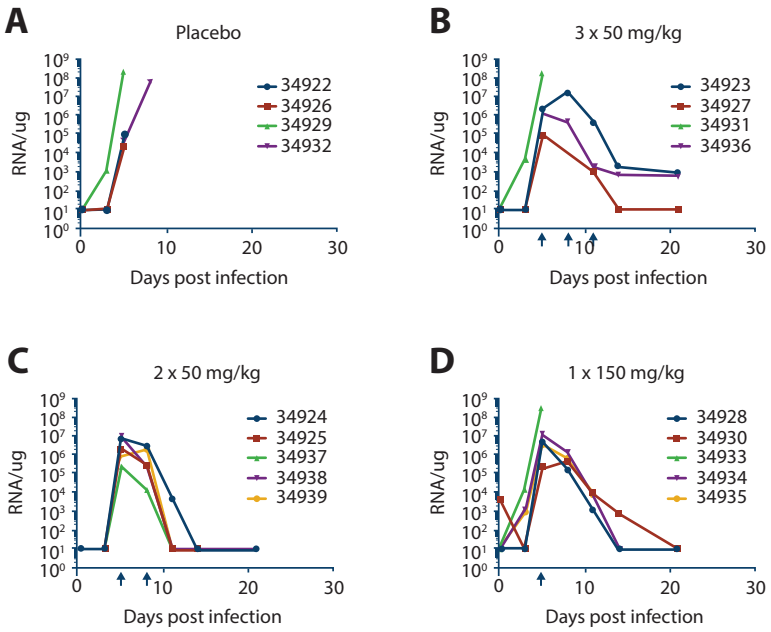


Note: (A) Survival plots, control/intervention, (B) viral load data for animals treated with placebo or with 3 doses mAbs mixture; plaque forming units/ml (pfu).

Blood samples were tested for viral load which means assessing the amount of virus in each sample. Known as viral load studies, the outcomes of the studies confirmed that antibody treatment was better at treating EBOV infection than the placebo. The mAbs therapeutic mixture reduced the amount of virus by over $1x10^6$ (1,000,000) with a 100% survival rate.

A major challenge with EBOV is a timely diagnosis. Initial symptoms of EBOV may not lead people to seek treatment. Because of anticipated delays in seeking medical treatment, a therapeutic intervention needs to effectively treat those who already have the EBOV infection. People infected with EBOV show a significant temperature spike on day 5 which is when they are most likely to seek medical treatment. For this reason, scientists sought to test the effectiveness of the 3 mAbs mixture at 5 days post infection. They also sought to determine the optimal dosage to treat EBOV (figure 4).

Figure 4: Dosage Impact on EBOV RNA Levels



Note: Using a control/intervention model scientists tested the impact of different dosage protocols at 5 days post infection on NHPs. Treatments for intervention groups, graphs B, C, and D, started on day 5. Data in graph B represents outcomes with three doses of 50 mg/kg were administered. Graph C represents outcomes with two doses of 50 mg/kg and graph D shows outcomes from one dose on day 5 at 150 mg/kg.

Discussion

In this study, scientists developed a therapeutic mixture of fully human antibodies to treat EBOV with the goal to 1) improve efficacy/outcomes for infected patients, 2) reduce resistance of EBOV to treatment, and 3) increase the potential to effectively treat outbreaks of EBOV and use the therapeutic to develop a vaccine to prevent EBOV outbreaks.

Scientists used conventional and novel approaches to test various fully human antibodies for efficacy with EBOV. The resulting data identified three antibodies whose mechanisms and non-competing epitopes proved most effective at bonding with surface GPs. Manufacturing processes were developed to efficiently produce mAbs, creating an effective therapeutic based on data evidence in both in vitro and in vivo studies. The results of this study have resulted in approved clinical trials on healthy volunteers as a preventative intervention for future EBOV outbreaks. The innovative methods developed during this study are being used to develop therapeutics for viruses such as Zika virus and for future emerging viral threats.