

## EBOLA

# Therapeutic Intervention of Ebola Virus Infection in Rhesus Macaques with the MB-003 Monoclonal Antibody Cocktail

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Ebola virus (EBOV) remains one of the most lethal transmissible infections and is responsible for high fatality rates and substantial morbidity during sporadic outbreaks. With increasing human incursions into endemic regions and the reported possibility of airborne transmission, EBOV is a high-priority public health threat for which no preventive or therapeutic options are currently available. Recent studies have demonstrated that cocktails of monoclonal antibodies are effective at preventing morbidity and mortality in nonhuman primates (NHPs) when administered as a post-exposure prophylactic within 1 or 2 days of challenge. To test whether one of these cocktails (MB-003) demonstrates efficacy as a therapeutic (after the onset of symptoms), we challenged NHPs with EBOV and initiated treatment upon confirmation of infection according to a diagnostic protocol for U.S. Food and Drug Administration Emergency Use Authorization and observation of a documented fever. Of the treated animals, 43% survived challenge, whereas both the controls and all historical controls with the same challenge stock succumbed to infection. These results represent successful therapy of EBOV infection in NHPs.

## INTRODUCTION

Since its discovery and initial characterization in the mid-1970s, Ebola virus (EBOV; formerly known as *Zaire ebolavirus*; genus: *Ebolavirus*, family: Filoviridae) has remained one of the most virulent and deadly pathogens known. With mortality rates approaching 90%, the virus quickly overwhelms the host, inducing a severe hemorrhagic fever and often death during sporadic outbreaks (1, 2). There are currently no licensed vaccines or therapeutics to prevent or treat infection with EBOV or any filovirus. With the increasing ease and speed of global travel and the potential for viral spread via the aerosol route (3), EBOV is a potential public health threat (4). Classification by the Centers for Disease Control as a category A agent also designates EBOV as a bioterrorism threat, making this virus a biodefense research priority (5).

Research has identified phosphorodiamidate morpholino oligomers (PMOs), small interfering RNAs (siRNAs), and a vesicular stomatitis virus (VSV)-based vaccine as potential candidates for post-exposure treatment (6–8). These candidates have shown promising efficacy in reducing mortality when administered to nonhuman primates (NHPs) up to 1 hour after exposure. More recently, antibodies were demonstrated to be highly effective in post-exposure prophylaxis of NHPs against EBOV. Passive transfer of macaque hyperimmune globulin was shown to protect rhesus macaques when dosing began 2 days after exposure (9). Similarly, a cocktail of three murine monoclonal antibodies (mAbs) provided 100 and 50% efficacy in cynomolgus macaques when dosing began 1 or 2 days after exposure, respectively (10). Final-

ly, a cocktail of three mAbs with human constant regions (MB-003) manufactured in *Nicotiana benthamiana* (11) provided 100 or 67% protection in the rhesus macaque model when treatment began 1 hour or 2 days after exposure, respectively, with no clinical indications of disease observed in survivors.

The development of MB-003 built upon previous work showing that mouse mAbs conferred protection up to 2 days after exposure in a murine challenge model (12). Three of these mAbs—13C6, 13F6, and 6D8—were protective individually, recognizing different non-overlapping EBOV glycoprotein epitopes, and these antibodies were subsequently deimmunized and/or chimerized with human constant regions to make them suitable for use in humans (that is, MB-003) (13). When tested in mice and in initial NHP studies, we found that *N. benthamiana*-derived MB-003 provided superior protection compared to Chinese hamster ovary-derived MB-003 (11). With efficacy in NHPs when administered 48 hours after exposure, these antibody post-exposure prophylactics may be useful for laboratory accidents where the agent and time of infection are known, but from a public health standpoint, treatment of the disease after the onset of symptoms and identification of the causative pathogen are priorities. Here, we present a concept study in the rhesus macaque model in which we observed significant protection using a therapeutic protocol where the administration of MB-003 was delayed until two “triggers,” a positive reverse transcription polymerase chain reaction (RT-PCR) result and fever resulting from EBOV challenge, were met.

## RESULTS

After intramuscular challenge with a target dose of 1000 plaque-forming units (PFU) (actual dose, 1067 PFU as determined by agarose plaque assay) of a well-characterized EBOV stock, animals were monitored via

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telemetry, and small samples of plasma were taken twice daily for RT-PCR analysis (Table 1). Viremia was initially detected via the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) Diagnostic Services Division (DSD) EZ1 rRT-PCR pre-EUA Assay Kit and quantified using a separate RT-PCR assay. Fever was noted remotely in all animals at varying time points using Data Sciences International (DSI) telemetry implants and software. Upon a positive RT-PCR test and a documented fever of  $\geq 1.5^{\circ}\text{C}$  above baseline (established before challenge) for 1 hour (six consecutive time points), treatment of the animals with MB-003 cocktail (50 mg/kg) was initiated. The limiting trigger in five of the seven animals was a fever. NHPs presented with a fever between 100 and 110 hours after exposure (Fig. 1), and RT-PCR for detection resulted in the positive detection of virus in all NHPs by the 111-hour post-exposure sample collection time point (Table 1). For all animals, the first treatment with MB-003 began by 120 hours after exposure, and the NHPs received two additional doses of MB-003 at about 170 and 250 hours after exposure.

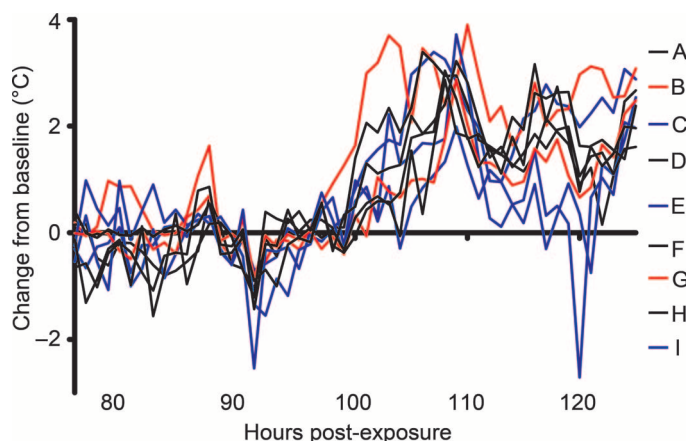
Both the phosphate-buffered saline (PBS) control (NHP B) and the irrelevant mAb-treated control [*N. benthamiana*-derived human anti-HIV immunoglobulin G1 (IgG1) mAb; NHP G] succumbed to challenge on days 7 and 11, respectively. These animals had a time to death consistent with historical controls using the same viral stock and presented with symptoms consistent with filoviral disease (Table 2). Four of the seven NHPs treated with MB-003 did not survive the infection, succumbing to challenge between days 7 and 12 with symptoms consistent with filoviral disease. In contrast, three of seven experimental animals survived challenge with varying levels of disease (Fig. 2,  $P = 0.029$  by Mantel-Cox against historic controls challenged intramuscularly with the same stock under similar infusion regimens). One (NHP E) of the three survivors developed a moderate rash and moderate prostration, whereas the others (NHP C and I) presented with no apparent symptoms of disease (Table 2). All animals had positive titers via quantitative RT-PCR (Fig. 2), although the survivors had noticeably lower levels of viremia at the time of treatment compared to the treated nonsurvivors. Virus was only detected by agarose plaque assay in the control animals (Fig. 2); the lack of plaques in samples from nonsurvivors has been reported in previous studies in which

MB-003 was administered, suggesting not only that there was sufficient mAb present to inactivate the virus (in the presence of complement) but also that there were no escape mutants present in the serum (11).

Notable differences between survivors and nonsurvivors were observed in whole blood and in the chemical analysis of blood samples collected during the course of the study (Fig. 3). Decreased platelet and red blood cell counts were observed in all NHPs succumbing to challenge, whereas nonsurvivors who received treatment exhibited elevated liver enzymes and substantial morbidity.

## DISCUSSION

This study extends our previous demonstration of post-exposure prophylaxis with MB-003 (11) to efficacy in a therapeutic protocol. This therapeutic study design sought to test intervention after two



**Fig. 1. Truncated telemetry analysis of NHPs.** Baseline temperatures for each NHP were determined before challenge. Changes in this baseline reading at each time point for each NHP were calculated and are graphically represented. NHPs first presented with fever between 100 and 110 hours after exposure; survivors are represented in blue, nonsurvivors in black, and controls in red.

**Table 1. Symptom-initiated treatment of EBOV infection.** NHPs were challenged intramuscularly with 1067 PFU of EBOV and monitored for clinical symptoms, fever, and the presence of viremia. Once NHPs

NHP	PCR trigger (hours after exposure)	Temperature trigger (hours after exposure)	Limiting trigger	Initial dosing (hours after exposure)	Treatment	Survival?
A	63	105	Temperature	108	MB-003	No (D9)
B	75	102	Temperature	104	Control (PBS)	No (D7)
C	87	108	Temperature	109	MB-003	Yes
D	63	103	Temperature	103	MB-003	No (D12)
E	111	104	PCR	120	MB-003	Yes
F	99	106	Temperature	108	MB-003	No (D8)
G	99	109	Temperature	113	Control (irrelevant mAb)	No (D11)
H	87	109	Temperature	113	MB-003	No (D10)
I	111	109	PCR	119	MB-003	Yes

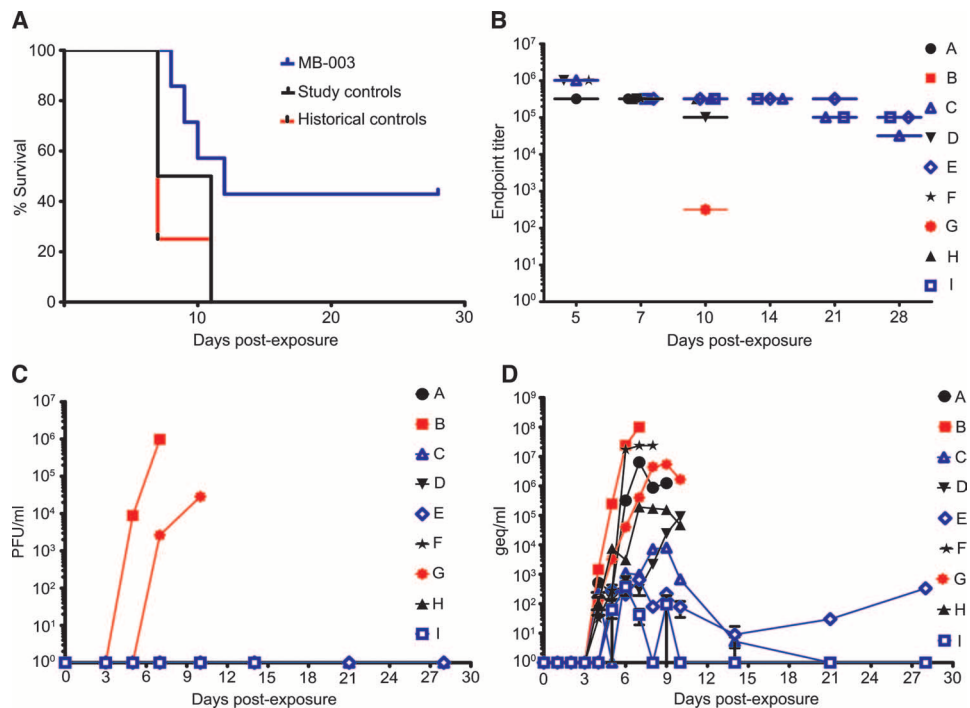
presented with both fever and a positive RT-PCR result, administration of MB-003 was initiated. Three of seven experimental NHPs survived challenge, whereas both control NHPs succumbed to challenge.

**Table 2. Clinical findings, days 0 to 28.** Thrombocytopenia was determined by Hemavet 950. Moderate petechial rash is defined as 10 to 40% of body surface area; severe petechial rash is defined as >40% of body surface area. AST, aspartate aminotransferase; ALT, alanine amino-

transferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CRE, creatinine; GGT,  $\gamma$ -glutamyl transpeptidase; TBIL, total bilirubin; NS, non-survivor; S, survivor.  $\uparrow$ , two- to threefold increase;  $\uparrow\uparrow$ , four- to fivefold increase;  $\uparrow\uparrow\uparrow$ , more than fivefold increase.

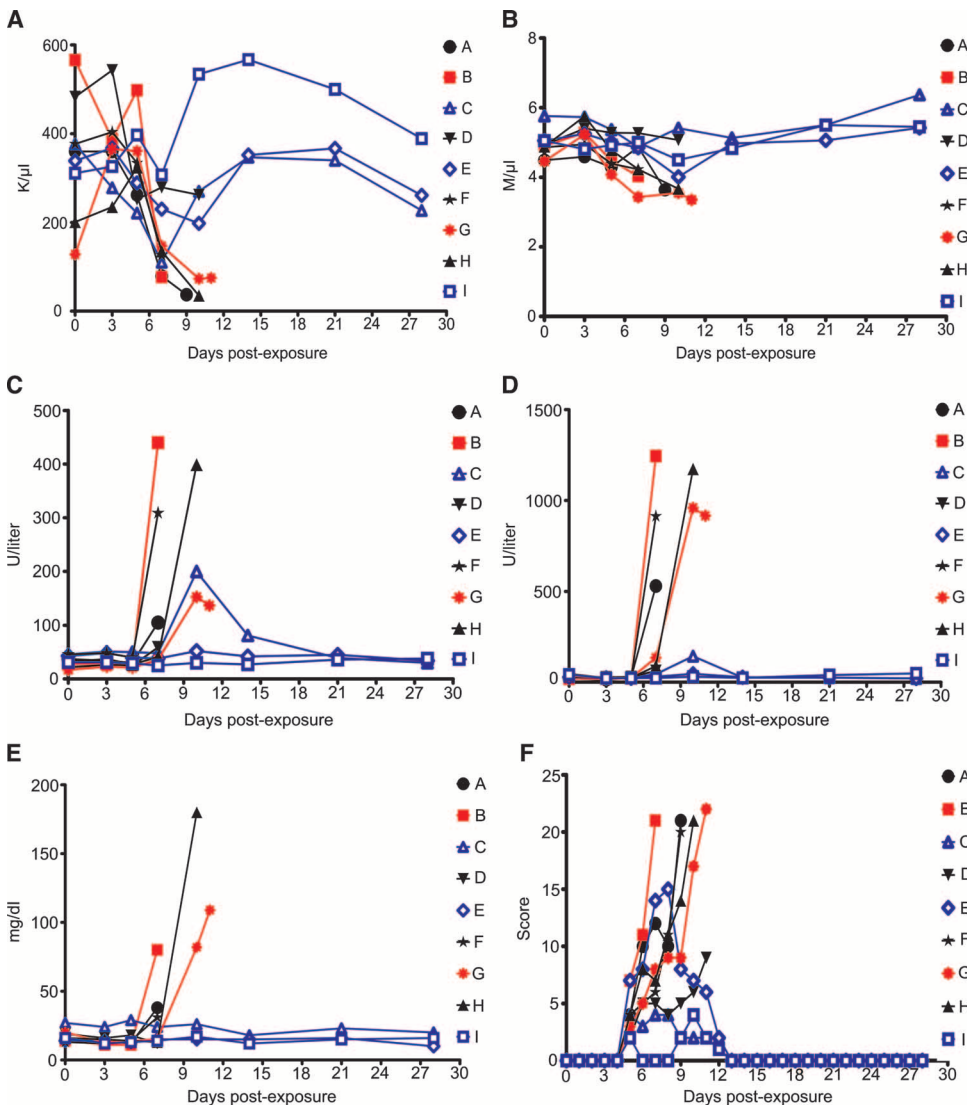
NHP	Treatment	Symptoms presented/clinical findings
A (NS)	MB-003	Thrombocytopenia, AST $\uparrow\uparrow\uparrow$ , BUN $\uparrow$ , ALT $\uparrow$ , CRE $\uparrow$ , ALP, severe petechial rash, bleeding, dehydration, increasing unresponsiveness, euthanized D9
B (NS)	PBS	Thrombocytopenia, BUN $\uparrow\uparrow\uparrow$ , CRE $\uparrow\uparrow\uparrow$ , ALT $\uparrow\uparrow\uparrow$ , AST $\uparrow\uparrow\uparrow$ , ALP $\uparrow\uparrow$ , GGT $\uparrow\uparrow$ , severe petechial rash, dehydration, increasing unresponsiveness, euthanized D7
C (S)	MB-003	Thrombocytopenia, ALT $\uparrow\uparrow$ , ALP $\uparrow$ , GGT $\uparrow$
D (NS)	MB-003	Thrombocytopenia, severe petechial rash, bleeding, some unresponsiveness, found dead D12
E (S)	MB-003	Thrombocytopenia, ALP $\uparrow$ , moderate petechial rash, unresponsiveness
F (NS)	MB-003	Thrombocytopenia, BUN $\uparrow$ , CRE $\uparrow$ , ALT $\uparrow\uparrow\uparrow$ , AST $\uparrow\uparrow\uparrow$ , ALP $\uparrow$ , GLU $\downarrow$ moderate petechial rash, dehydration, increasing unresponsiveness, euthanized D8
G (NS)	Irrelevant mAb	Thrombocytopenia, BUN $\uparrow\uparrow$ , CRE $\uparrow$ , ALT $\uparrow\uparrow\uparrow$ , AST $\uparrow\uparrow\uparrow$ , ALP $\uparrow\uparrow$ , GGT $\uparrow\uparrow$ , GLU $\downarrow$ moderate petechial rash, bleeding, increasing unresponsiveness, loss of balance, euthanized D11
H (NS)	MB-003	Thrombocytopenia, BUN $\uparrow\uparrow\uparrow$ , CRE $\uparrow\uparrow\uparrow$ , ALT $\uparrow\uparrow\uparrow$ , AST $\uparrow\uparrow\uparrow$ , TBIL $\uparrow$ , severe petechial rash, bleeding, dehydration, increasing unresponsiveness, euthanized D10
I (S)	MB-003	Thrombocytopenia, ALP $\uparrow$ , mild dehydration

diagnostic triggers: RT-PCR to assess detectable viral RNA (assay processing time is about 3 hours from collection) using the DSD RT-PCR protocol and a documented fever ( $+1.5^{\circ}\text{C}$  over baseline for  $\geq 1$  hour). Treatment was initiated (16.7 mg/kg per mAb) at the dose previously shown to provide post-exposure protection (11). In seven of nine animals, a positive RT-PCR test was observed before a documented fever. Whereas all animals developed a fever within a relatively narrow window during the course of infection (101 to 110 hours after exposure), the relative viral titer by RT-PCR varied significantly among NHPs at parallel time points. This observation may provide some insight into early-onset disease in addition to providing guidance for the treatment dose and dosing regimen. Indeed, NHPs that were slower to develop viral titers were much more likely to survive challenge; for the two animals where the RT-PCR trigger came after fever (Table 1, NHP E and I), treatment did not begin until about 120 hours after exposure; both of these animals survived challenge. It would be easy to conclude that treatment would have been more effective if initiated as soon as viremia was detected. The requirement of meeting both triggers in this study was likely more stringent than would be required for therapy during a known outbreak where detection of viremia would be sufficient to initiate treatment. Among the treated animals, 43% (three of seven) survived, whereas two of the



**Fig. 2. Survival, mAb titers, and viral levels during the therapeutic protocol.** (A) MB-003 provided 43% protection (three of seven) when used as a therapeutic against EBOV infection ( $P = 0.0291$ , log-rank Mantel-Cox;  $n = 1$ ) compared with four of four historical controls infected by the same route, challenge dose, and stock and infusion regimen. (B) Total anti-EBOV glycoprotein antibody titers were determined by enzyme-linked immunosorbent assay (ELISA). (C and D) Viremia was assessed by agarose plaque assay (C) and quantitative RT-PCR (D). Survivors are represented in blue (open symbols), nonsurvivors in black, and controls in red. geq, genomic equivalence.

two study controls (one received PBS and one received an irrelevant *N. benthamiana*-derived isotype control) and four of the four historic controls challenged with the same stock, target dose, and challenge



**Fig. 3. Clinical analysis and observations for NHPs.** Complete blood counts (CBCs) and chemical analyses were performed on samples collected over the course of the study (days 0, 3, 5, 7, 10, 14, 21, and 28). (A and B) Among nonsurvivors, thrombocytopenia (A) and anemia (B) were observed. (C and D) In most experimental nonsurvivors and both controls, elevated levels of aspartate aminotransferase (AST; C) and alanine aminotransferase (ALT; D) were observed. (E) Blood urea nitrogen assessment revealed elevated levels in most nonsurvivors. (F) All nonsurvivors had progressively more severe morbidity in daily observation scores. Survivors are represented in blue (open symbols), nonsurvivors in black, and controls in red.

route and receiving the same infusion regimen succumbed to infection. Two of three surviving animals displayed minimal disease symptoms, whereas the third one exhibited moderate symptoms of infection.

RT-PCR viremia data suggest that viral titers stabilize and drop, resulting in survival, when treatment was initiated before a threshold of about  $10^4$  genomic equivalence; more frequent dosing may potentially keep the titer stabilized or low enough to give the adaptive immune response time to supplement the treatment. The RT-PCR titer of one survivor (Fig. 2, NHP E) slowly began to rise by the conclusion of the study, which was terminated on day 28 as per the Institutional Animal Care and Use Committee (IACUC) protocol. Because no virus was detected in serum by plaque assay, we believe it was unlikely that an escape

mutant was present. Because CD8<sup>+</sup> T cells have been shown to play a critical role in recovery from an Ebola exposure (14, 15), this NHP may have had a poor T cell response, which allowed the virus to return as protective antibody titers declined.

Potent therapies for viral diseases such as EBOV may require combinational approaches to achieve optimal protection. Studies with adjuvants, other small-molecule antivirals, or supportive care such as fluid management and control of secondary infections may significantly improve the overall therapeutic outcome observed in this study.

The prominent role of antibodies in the immune response is well documented, and the use of mAbs in the treatment of infection is expanding. Furthermore, with the approval of the first plant-derived recombinant protein (16) in 2012 and the variety of *Nicotiana*-derived products in phase 1 and 2 clinical trials, plant-based manufacturing is becoming a viable platform with appealing speed and scalability (17). The ability to treat at a later time point after initial exposure and to mitigate further morbidity and disease pathogenesis underscores the therapeutic potential of mAbs in general and MB-003 specifically.

## MATERIALS AND METHODS

### Study design

Nine rhesus macaques were given telemetry implants and central venous catheters before entry into containment. Animals were randomized with Microsoft Excel, but the study was not blinded to study personnel. Seven NHPs were designated for treatment with MB-003 after the pre-established triggers of positive viremia (via RT-PCR) and elevated temperature ( $\geq 1.5^\circ\text{C}$  above an individually established baseline for  $\geq 1$  hour) were met. Controls

received an infusion of PBS ( $n = 1$ ) or an anti-HIV *N. benthamiana*-derived mAb ( $n = 1$ ) after the same triggers were met.

### Production of MB-003 mAbs in $\Delta\text{XT}/\text{FT } N. benthamiana$

To manufacture the MB-003 mAbs in plants, the “magnification” procedure was used as described previously (18) with minor modifications. Briefly, plants grown for 24 to 26 days in an enclosed growth room at  $22^\circ$  to  $24^\circ\text{C}$  were used for vacuum infiltration. Equal volumes of *Agrobacterium* cultures grown overnight were mixed with infiltration buffer [10 mM MES (pH 5.5) and 10 mM  $\text{MgSO}_4$ ], resulting in a 1:1000 dilution for each individual culture. With a 300-liter custom-built (Kentucky BioProcessing) vacuum chamber, the aerial parts of entire



plants were inverted into the bacteria/buffer solution, and a vacuum of 22 inches of mercury was applied for 2 min. At 7 days after infiltration, leaf tissues were extracted with a Corenco double-stack disintegrator at a 0.5:1 buffer-to-plant tissue ratio [extraction buffer: 100 mM tris, 40 mM ascorbic acid, 1 mM EDTA (pH 8.5)]. The extract was adjusted to pH 8.0 with 10 M NaOH and then clarified with a plate frame filter press (ErtelAlsop) with 1.0- $\mu$ m pads. The antibody was captured from the filtrate with a MabSelect SuRe (GE Healthcare) protein A column. The column was equilibrated and washed with tris running buffer, and antibodies were eluted with acetic acid. The resulting eluate was neutralized with 1 M tris, and the mAb solution was further purified via Q filtration (Mustang Q membrane; Pall). The final polishing column for c13C6 and c6D8 was a CHT type I 40- $\mu$ m (Bio-Rad) column. The column was equilibrated and washed with phosphate running buffer, and antibodies were eluted with running buffer containing NaCl. The final polishing column for h-13F6 was an MEP HyperCel column equilibrated and washed with Hepes buffer, washed a second time at a slightly acidic pH to remove product-related impurities, and eluted under acidic conditions. The eluate was neutralized to pH 7.0. The final eluates for each antibody were concentrated and diafiltered against their respective formulations [c6D8: 50 mM sodium phosphate, 100 mM NaCl, 200 mM arginine, and 4% (w/v) mannitol; h-13F6 and c13C6: 50 mM sodium phosphate, 200 mM glycine, 8% (w/v) mannitol, and 0.005% polysorbate 20] with tangential flow ultrafiltration with 30-kD molecular weight cutoff polyethersulfone membranes. All three antibodies were sterile-filtered, aseptically aliquoted into glass vials with serum stoppers, sealed, and stored at  $-80^{\circ}\text{C}$ . According to SDS-polyacrylamide gel electrophoresis analysis, the mAbs were fully assembled with less than 5% aggregation, as determined by high-performance liquid chromatography/size-exclusion chromatography. An Ebola glycoprotein antigen-binding ELISA was used to confirm binding activity compared to reference standards.

### Virus stocks

EBOV (Zaire Kikwit strain) stocks were developed at the USAMRIID with virus originally isolated from an infected patient during the 1995 outbreak and passaged in Vero E6 cells. The stock material for the study was obtained in four passages from the original isolate. This stock has been deemed the national stock for preclinical studies for advanced products. The stock was made by the Department of Defense Critical Reagents Program under the Joint Program Executive Office for Chemical and Biological Defense. The virus and future viruses generated under this program have been developed under the Filovirus Animal Non-Clinical Group (FANG), a multiple-agency group including the U.S. Food and Drug Administration, to prepare for validated/regulated efficacy studies.

### NHP challenge and care

Adult male and female rhesus macaque NHPs were caged individually. NHPs underwent surgery for TA10TA-D70 telemetry implantation (DSI). After the NHPs were placed in training jackets (Lomir Biomedical) for acclimatization, which lasted for 6 days, surgeries to place central venous catheters (CVCs) (Groshong 7F; Bard) were performed, and adequate recovery time was provided before transfer into biosafety level 4 (BSL4) containment. After CVC placement, custom jackets (Lomir Biomedical) were used to contain and protect the CVC lines connected to a mounted swivel system (Instech Solomon). These lines were locked with taurolidine-citrate catheter solution (UNO B.V.). After the NHPs

were moved into containment, acclimated, and connected to the swivel system, the lines were flushed at least once daily with PBS (BD) and locked with heparin (BD) to maintain line functionality. NHPs were given monkey chow (Harlan), primate treats, fruits, and vegetables for the duration of the study. Before challenge, NHPs were anesthetized and given a physical examination. Animals were challenged intramuscularly with a target dose of 1000 PFU/ml diluted from the stock concentration with minimum essential medium (MEM). After challenge, animals were returned to their cages and observed until reasonable mobility was recovered.

### Verification of target dose and viremia

Target challenge doses and viremia were verified by agarose-based plaque assay. Dilution points were serially diluted 10-fold in Eagle's MEM and adsorbed onto Vero E6 cell monolayers in six-well plates. The plates were incubated for 1 hour at  $37^{\circ}\text{C}/5\% \text{CO}_2$  with rocking about every 15 min, and 2 ml of a 1:1 mixture of medium [2 $\times$  Eagle's basal medium with Earle's salts, 10% (v/v) fetal bovine serum] and 1% agarose (Lonza) was added. Plates were then incubated for 7 days at  $37^{\circ}\text{C}/5\% \text{CO}_2$ . After incubation, 2 ml of a 1:1 mixture of the same medium and 4% neutral red stain was added to each well. After an additional 24 hours of incubation at  $37^{\circ}\text{C}/5\% \text{CO}_2$ , the plaques were counted, and viremia titers were calculated. Viremia analysis was performed on plasma samples with the same methodology.

### MB-003 preparation and treatment

Treatments consisted of a mixture of three mAbs, and each mAb was equally represented in the treatment mixture. Treatment for each group was set at 16.7 mg/kg per mAb. The treatment for each specific NHP was determined according to the animal's weight before challenge. NHPs were treated via intravenous infusion through the CVC with 60-ml syringes (BD) and syringe pumps (Lomir Biomedical) over about 280 min. NHPs received MB-003 (50 mg/kg). After the initial positive triggers, all NHPs were treated every 3 days. In total, three infusions were given to each NHP.

### Animal monitoring and sample collection

NHPs were monitored for changes in health, diet, behavior, and appearance. Samples used for RT-PCR analysis were collected twice per day at 12-hour increments; if anesthesia was required for collection, samples were only collected once daily in the morning. Samples used for CBCs and chemistry analyses were collected on days 0, 3, 5, 7, 10, 14, 21, and 28. CBCs were performed with the Hemavet 950 (Drew Scientific), and chemistry analyses were performed with Piccolo 13 general chemistries (Abaxis). All blood collections were performed with 1-ml minicollect serum and K3 EDTA plasma tubes (Greiner Bio-One). All sample tubes were centrifuged at 1800g for 10 min, and the resulting material was used or frozen at  $-80^{\circ}\text{C}$  for further analysis. NHP temperatures were monitored remotely via DSI telemetry every 10 min with the corresponding software (DSI). Experiments were conducted under BSL4 containment conditions.

### Enzyme-linked immunosorbent assay

ELISAs were performed with a recombinant EBOV glycoprotein (National Cancer Institute), and plasma samples were serially diluted at half-log increments. Goat anti-human IgG (heavy + light) (KPL) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Millipore) were used as secondary antibodies and substrate, respectively.

Assays were evaluated at an absorbance at 405 nm (SpectraMax M5; Molecular Devices).

### RNA isolation and analysis

Qualitative real-time RT-PCR (rRT-PCR) was used for the detection of Ebola Zaire virus in plasma samples from exposed NHPs. Samples from the NHPs were inactivated with TRI reagent LS (at a ratio of three parts of TRI Reagent LS to one part of plasma). RNA in each sample was purified with the Qiagen QIAamp Viral RNA Mini Kit. A volume of 70  $\mu$ l of inactivated plasma was placed onto the column, and samples were eluted in 70  $\mu$ l of AVE buffer provided by Qiagen. The eluted RNA was run in triplicate with the USAMRIID DSD EZ1 rRT-PCR pre-EUA Assay Kit on the ABI 7500 Fast Dx instrument. This assay kit uses SuperScript II One-Step rRT-PCR sequence-specific primers and a hydrolyzable probe (TaqMan) against a conserved region of the Ebola Zaire virus. The PCR cycling conditions were as follows: reverse transcription for 15 min at 50°C, Taq activation for 5 min at 95°C, and 45 cycles of amplification with a denaturation step for 1 s at 95°C and an anneal/extension step for 26 s at 60°C. Samples were run immediately for qualitative detection at two time points on days 3 to 5 after infection, with a.m. and p.m. time points where applicable. The sample was considered positive if one of the three replicate reactions was detected with a  $C_t$  value or negative if all three replicate reactions were undetected. The time required from sample receipt to reportable detection was about 3 hours.

### Quantitative RNA isolation and RT-PCR

RNeasy kits (Qiagen) were used for RNA extraction. One-step quantitative real-time RT-PCRs were performed with a LightCycler 480 (Roche) in 20- $\mu$ l volumes with 5  $\mu$ l of purified RNA using the SuperScript III One-Step RT-PCR System (Invitrogen). Primers (forward, 5'-CGGA-CCTGGTTGGTTGTG-3'; reverse, 5'-GCTGCAGTGTTCGCATCTGA-3') and TaqMan probe (6-carboxyfluorescein-5'-CCCTTGCCACAATCT-minor groove binder nonfluorescent quencher-3') (Applied Biosystems) specific for the Ebola Zaire glycoprotein gene were used. Program conditions consisted of reverse transcription at 50°C for 20 min and initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 5 s; annealing, synthesis, and signal acquisition at 60°C for 20 s; and a final cooling step at 40°C for 30 s. Measurements of viral gene expression were performed with a viral RNA standard.

### Statistics

Survival curves were analyzed with the log-rank Mantel-Cox test. For each test,  $P < 0.05$  was considered statistically significant. Survival data are consistent with proportional hazards.

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## Therapeutic Intervention of Ebola Virus Infection in Rhesus Macaques with the MB-003 Monoclonal Antibody Cocktail

James Pettitt, Larry Zeitlin, Do H. Kim, Cara Working, Joshua C. Johnson, Ognian Bohorov, Barry Bratcher, Ernie Hiatt, Steven D. Hume, Ashley K. Johnson, Josh Morton, Michael H. Pauly, Kevin J. Whaley, Michael F. Ingram, Ashley Zovanyi, Megan Heinrich, Ashley Piper, Justine Zelko and Gene G. Olinger

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### Better Late than Never

They say prevention is better than a cure, but sometimes, immediate action isn't possible. This is especially the case for a deadly disease such as Ebola virus (EBOV) infection, where sporadic outbreaks make it hard to predict when and where treatment will be needed. In patients, neither preventative nor therapeutic options are currently available, but recent studies have shown that a cocktail of monoclonal antibodies may help if given within 1 to 2 days of challenge in macaques. Pettitt *et al.* now extend this window, showing that this antibody cocktail can be used as a therapeutic in nonhuman primates (NHPs) even after the onset of symptoms.

The authors challenged NHPs with EBOV and didn't begin treatment until after confirmation of infection and observation of fever. Although the controls here and all historical controls succumbed to infection, 43% of the treated animals survived the challenge. If these observations hold true in humans, these monoclonal antibodies could give hope to people exposed to EBOV.

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