CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761169Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

Integrated Review

Table 1. Administrative Application Information

Category	Application Information
Application type	BLA
Application number(s)	BLA 761169
Priority or standard	Priority
Submit date(s)	2/25/2020
Received date(s)	2/25/2020
PDUFA goal date	10/28/2020
Division/office	Division of Antivirals (DAV)
Review completion date	10/14/2020
Established name	Atoltivimab, maftivimab, and odesivimab-ebgn
(Proposed) trade name	Inmazeb
Pharmacologic class	Antiviral/Ebolavirus (7030249)
Code name	Atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479); REGN3470-3471-3479; REGN-EB3
Applicant	Regeneron Pharmaceuticals, Inc.
Dose form/formulation(s)	Solution for injection
Dosing regimen	The recommended dosage of INMAZEB is 50 mg of atoltivimab, 50 mg of maftivimab, and 50 mg of odesivimab per kg (3 mL/kg) diluted and administered as a single intravenous infusion. Throughout the review we refer to INMAZEB dosing as 150 mg/kg as a single intravenous infusion
Applicant proposed indication(s)/population(s)	Treatment of infection caused by Zaire ebolavirus
Proposed SNOMED indication	37109004 | Ebola virus disease (disorder)
Regulatory action	Approval
Approved indication(s)/population(s) (if applicable)	INMAZEB is indicated for the treatment of infection caused by Zaire ebolavirus in adult and pediatric patients, including neonates born to a mother who is RT-PCR positive for Zaire ebolavirus infection. Limitations of Use The efficacy of INMAZEB has not been established for other species of the Ebolavirus and Marburgvirus genera. Zaire ebolavirus can change over time, and factors such as emergence of resistance, or changes in viral virulence could diminish the clinical benefit of antiviral drugs. Consider available information on drug susceptibility patterns for circulating Zaire ebolavirus strains when deciding whether to use INMAZEB.
Approved SNOMED indication	37109004 | Ebola virus disease (disorder)

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Glossary

ADA	antidrug antibody
ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
APT	all patients treated
AR	adverse reaction
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC_{inf}	AUC from time zero extrapolated to infinity
BLA	biologics license application
CBER	Center for Biologics Evaluation and Research
CFR	Code of Federal Regulations
C_{max}	maximum plasma concentration
CMC	chemistry, manufacturing, and controls
CRF	case report form
CSR	clinical study report
Ct	cycle-threshold
CtGP	cycle-threshold glycoprotein gene targets
CtNP	cycle-threshold nucleoprotein gene targets
DAV	Division of Antivirals
DRC	Democratic Republic of the Congo
DSMB	data safety monitoring board
EAP	expanded access protocol
EBOV	*Zaire ebolavirus*
EC_{50}	half-maximal effective concentration
EM	electron microscopy
ETU	Ebola treatment unit
EVD	*Zaire ebolavirus* disease
FDA	Food and Drug Administration
FDIC	found dead in cage
GCP	good clinical practices
GE	genome equivalent
GP	glycoprotein
HDX-MS	hydrogen-deuterium exchange coupled to mass spectrometry
IC_{50}	half-maximal inhibitory concentration
ICF	informed consent form
IM	intramuscularly
IND	investigational new drug
IP	intraperitoneal
IRB	Institutional Review Board
ITT	intention-to-treat

IV	intravenous
K_D	equilibrium dissociation constant
KM	Kaplan-Meier
LLOQ	lower limit of quantitation
LOD	limit of detection
mAb	monoclonal antibody
MEURI	Monitored Emergency Use of Unregistered and Investigational Interventions
MedDRA	Medical Dictionary for Regulatory Activities
MOA	mechanism of action
MSF	Médecins Sans Frontiéres
NDA	new drug application
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NP	nucleoprotein
oSOC	optimized standard of care
PALM	PAmoja TuLinde Maisha
PD	pharmacodynamics
PFU	plaque-forming unit
PK	pharmacokinetics
PLLR	Pregnancy and Lactation Labeling Rule
PMC	postmarketing commitment
PMR	postmarketing requirement
PREA	Pediatric Research Equity Act
PRNT	plaque reduction neutralization titer
REGN3470	atoltivimab
REGN3471	odesivimab
REGN3479	maftivimab
REGN-EB3	atoltivimab, maftivimab, and odesivimab-ebgn
RT-PCR	reverse transcription-polymerase chain reaction
SAE	serious adverse event
sGP	secreted glycoprotein
SNP	single-nucleotide polymorphism
SOP	standard operating procedure
SPR	surface plasmon resonance
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
VLP	virus-like particle
VSV	vesicular stomatitis virus
WHO	World Health Organization

I. Executive Summary

1. Summary of Regulatory Action

This new Biologics License Application (BLA) for INMAZEB, a combination of three human recombinant IgG1κ monoclonal antibodies (mAbs) (atoltivimab, maftivimab, and odesivimab) each targeting the *Zaire ebolavirus* (EBOV) glycoprotein, was submitted by Regeneron Pharmaceuticals, Inc. The BLA was reviewed by a multidisciplinary team. The intended indication is for the treatment of infection caused by EBOV in adult and pediatric patients, including neonates born to a mother who is positive by reverse transcriptase-polymerase chain reaction for EBOV infection. INMAZEB (referred to as REGN-EB3 throughout the review) is the first product to be approved for the treatment of EBOV infection.

The regulatory history is notable for Orphan Drug designation and Breakthrough Therapy designation. This BLA received a Priority Review and was not presented at the Antimicrobial Drugs Advisory Committee because REGN-EB3 received Breakthrough Designation and the benefit-risk assessment was not controversial based on the review team's preliminary assessment of the trial results.

Each discipline (Clinical, Clinical Virology, Clinical Pharmacology, Pharmacology/Toxicology, Statistics and Regulatory) did not identify any issues that preclude approval. I, the signatory authority, agree that the benefit-risk assessment favors approval.

Originally, the development program for REGN-EB3 was based on fulfilling the necessary criteria for potential approval under the Animal Rule pathway. However, when a new outbreak of EBOV infection was declared in the Democratic Republic of the Congo (DRC), an expanded access protocol for emergency use was implemented followed by the initiation of the PAmoja TuLinde Maisha (PALM) trial by the National Institute of Allergy and Infectious Diseases and the Institut National de Recherche Biomédicale (INRB) of the DRC with support from other donors. The nonhuman primate challenge studies in rhesus macaques along with the phase 1 data in healthy volunteers provided the basis to evaluate a single dose of 50 mg/kg of each mAb (total 150 mg/kg) in the PALM trial.

The PALM trial compared three investigational agents (two mAb products and one small molecule) to an investigational control ZMapp (another mAb). The use of ZMapp as the investigational control arm was deemed acceptable by the review team based on the trials results from the PREVAIL II trial, local health authority preference, and the superiority trial design (review issue discussed in Section II.6.3.1).

The results of the PALM trial clearly demonstrated efficacy to support the approval of REGN-EB3 for the treatment of adult and pediatric patients infected with EBOV. The PALM trial was stopped early on the basis of a prespecified interim analysis showing a significant reduction in

mortality for REGN-EB3 (34%) compared to control (51%). The results from this single trial are adequate to support approval because of the significant results. However, lower efficacy was seen in subjects with a cycle-threshold nucleoprotein gene target value of ≤22 (CtNP ≤ 22; which correlates with a higher viral load) versus those with a value of >22. Although the PALM study demonstrated REGN-EB3 was efficacious, some uncertainties remain, including whether a higher dose of one or all three mAbs is needed for an optimally efficacious dose in patients with high baseline viral loads (review issue discussed in Section II.6.3.2). A postmarketing commitment was issued to evaluate the efficacy, safety, and pharmacokinetics of a higher dose of REGN-EB3 versus REGN-EB3 150 mg/kg in adult and pediatric patients with cycle-threshold nucleoprotein gene target values of ≤22.

Based on the data submitted, REGN-EB3 has a favorable safety profile. Although some clinical assessments were limited by the challenging circumstances at the study sites, the safety database is sufficient for the evaluation of risk. Having met the primary efficacy objective, superiority in reduction of 28-day mortality, a degree of uncertainty in describing the risk attributable to REGN-EB3 was considered acceptable. Infusion-associated events, such as hypotension, chills, and elevation of fever, were reported peri- and post-infusion. The WARNINGS AND PRECAUTIONS section included a description of the potential for hypersensitivity reactions and recommendations for monitoring and mitigation of infusion-related reactions. The evaluation of adverse events in subjects who received REGN-EB3 may have been confounded by the signs and symptoms of the underlying EBOV infection. The most common adverse events reported in at least 20% of subjects were pyrexia (or elevation in fever), chills, tachycardia, tachypnea, and vomiting. Overall, the adverse event profile in adult and pediatric subjects treated with REGN-EB3 was similar.

The resistance pathway for each mAb was not characterized and no human resistance data were available from the PALM trial. Two postmarketing requirements were issued to characterize the resistance profile.

As stated, REGN-EB3 is a combination of three mAbs; therefore, per 21 CFR 300.50, evidence is needed to show the contribution of each component to the combination. In the absence of clinical studies designed to evaluate the activity of each mAb versus the combination, other evidence is needed to satisfy 21 CFR 300.50. In this scenario components of the combination cannot be administered individually because of the highly lethal nature of EBOV infection and the possibility for rapid development of resistance if each mAb were administered individually. Section II.5.1 highlights the nonclinical assessment of potential effectiveness. The Applicant has fulfilled the requirements of 21 CFR 300.50 and provided nonclinical data to indicate that each mAb in the combination has activity. Some uncertainties remain with respect to the exact mechanism of action of each mAb and defining the precise epitopes for each mAb and resistance pathways. Postmarketing requirements and commitments were issued to address this issue.

Another review issue related to the evaluation of benefit is the lack of clinical data for the treatment of EBOV infection acquired by routes other than natural transmission. The PALM trial and the expanded access protocol treated subjects presumably infected by the natural transmission route (i.e., contact with infected blood or other bodily fluid). The nonclinical

studies did not model other routes of infection, such as aerosol or needlestick exposure. The Clinical Virology team's position is that the indication should state *naturally acquired* infection given that a needlestick exposure, which may involve a markedly greater inoculum, was not studied and the disease course is likely to be significantly different in the event of an intentional release. The Clinical review team agreed that the nonhuman primate studies were inadequate to demonstrate evidence of efficacy in the setting of needlestick or intentional release; however, restricting to *naturally acquired* infection could result in delay or deferral of use in these circumstances despite evidence that there may be some benefit in the context of needlestick exposure or other healthcare-associated exposures. The signatory concurs with the Clinical review team. Therefore, the indication does not reference route of infection and remains treatment of infection caused by EBOV.

Based upon review of all available efficacy and safety data, the benefits of REGN-EB3 clearly outweigh the risks for treatment of EBOV. The availability of REGN-EB3 will provide the first effective treatment option for adult and pediatric patients, including neonates and pregnant individuals, infected with EBOV.

For detailed information supporting the basis for the benefit-risk assessment please refer to the details in this integrated assessment document.

2. Benefit-Risk Assessment

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Ebolaviruses are negative-sense, single-stranded, RNA viruses that belong to the Filoviridae family and cause sporadic outbreaks in regions of western and equatorial Africa (Burrell et al. 2017). *Zaire ebolavirus* (EBOV) is one of four *Ebolavirus* species, which are highly pathogenic and can cause severe systemic and potentially fatal disease in humans and nonhuman primates (NHPs) (CDC 2019b). • Although all outbreaks to date have originated in western and equatorial Africa, the disease can spread internationally due to the ease of travel. Humans likely become infected with EBOV by handling sick or dead infected forest animals, and secondary human-to-human transmission typically occurs by exposure to blood or other infected bodily fluids through abraded skin or mucosal tissues. During the 2014 to 2016 outbreak, 11 individuals with EBOV infection were treated in the United States, 9 of whom had contracted it in western Africa, most as health care workers. Two American nurses contracted the disease in the US, and both recovered (CDC 2020). • The incubation period varies between 2 and 21 days with an average period of 6 to 12 days (Schieffelin et al. 2014). Early symptoms may include fever, myalgias, chills, and general malaise; followed by gastrointestinal symptoms such as diarrhea, vomiting, and abdominal pain (Malvy et al. 2019). Bleeding, manifested by petechiae, ecchymoses, and melena, occurs in less than 50% of laboratory-confirmed cases (Bwaka et al. 1999). Rapid deterioriation over the following 2 to 3 days is marked by onset of shock and multiorgan failure. Death usually occurs 6 to 9 days after onset of clinical disease, at which time patients typically are highly viremic and present a high risk of contagion (Burrell et al. 2017). • The avergage case fatality rate is around 50% but varies by outbreak, treatment setting, and availability of optimzed supportive care. Mortality rates have ranged from 25% to 90% (WHO 2020b).	*Zaire ebolavirus* causes a highly contagious infection with a rapidly progressive and often fatal clinical course. Outbreaks have originated in western and equatorial Africa, some of which have spread internationally. Preparedness has required extensive international collaboration and effort to rapidly detect and respond to new outbreaks.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current Treatment Options	• Investigational drugs for the treatment of EBOV infection have been studied in prior outbreaks; however, there are currently no approved antiviral drugs for the treatment of EBOV infection. • A recombinant vesicular stomatitis virus expressing the EBOV glycoprotein (rVSV-ZEBOV) vaccine (tradename "Ervebo") was approved for prevention of EBOV infection on December 19, 2019. Ervebo is a single-dose vaccine regimen that was found to be safe and protective against only the *Zaire ebolavirus* species of *Ebolavirus*. This was the first approval by the FDA of a vaccine for EBOV. • Standard of care is supportive and includes basic interventions, such as providing intravenous (IV) fluids, repletion of electrolytes, and as needed oxygen therapy, vasopressors, analgesics, antipyretics, antidiarrheals and antiemetics. Antibacterials and antimalarials are also often provided for suspected or confirmed concomitant infections.	Although a vaccine has recently been approved for prevention of *Zaire ebolavirus* infection, there are currently no approved antiviral drugs for treatment of EBOV infection. Standard of care is supportive. Antiviral treatments are needed in addition to vaccines when new outbreaks occur, where vaccination programs have not yet been deployed, and cases in which vaccination was not fully protective.

Benefit	• Nonclinical data show that all three monoclonal antibodies (mAbs) bind with high affinity to glycoprotein (GP) simultaneously and sequentially, with odesivimab (REGN3471) also binding to secreted glycoprotein (sGP), and that each mAb has antiviral activity. • Given the challenges of conducting adequate and well-controlled trials for treatment of EBOV infection, the development program for REGN-EB3 was initially based on fulfilling the necessary criteria for potential approval under the Animal Rule pathway. Nonhuman primate (NHP) studies were conducted in rhesus macaques challenged with a lethal dose of EBOV. NHPs given all doses of REGN-EB3 survived at a higher rate than those that received placebo. Together with initial phase 1 data in healthy humans, results of the NHP challenge studies provided a rationale for the single dose of 50 mg/kg of each antibody (combined dose of 150 mg/kg). • Emegency use of REGN-EB3 was implemented when a new outbreak of EBOV infection was declared in the North Kivu province of the Democratic Republic of the Congo (DRC) on August 1, 2018. An Expanded Access Protocol for Emergency Use (EAP 1846) was initiated in collaboration with the World Health Organization (WHO) and the Institut National de Recherche Biomedical (INRB) in the DRC using the Monitored Emergency Use of Unregistered Interventions (MEURI) framework allowing for the rapid introduction of investigational treatments during the outbreak. EAP 1846 helped to estimate mortality rates for patients treated with REGN-EB3. • PAmoja TuLinde Maisha (protocol 19-I-0003, or PALM), was initiated by the National Institute of Allergy and Infectious Diseases (NIAID) and an Agency of the DRC on November 20, 2018 as a three-arm randomized controlled trial (RCT). Based on clinical trial experience from the 2014 to 2016 West African epidemic, ZMapp (another investigational mAb cocktail) was chosen as a comparator in the PALM clinical trial during the 2018 eastern DRC outbreak. Three investigational treatments, including REGN-EB3, were compared to ZMapp. REGN-EB3 was added as a fourth arm in the third amendment of the protocol, and enrollment into the REGN-EB3 arm started on January 26, 2019. • The intial phase of the PALM trial was stopped early based on an interim analysis that demonstrated superiority for the primary	REGN-EB3, when given as a single dose of 150 mg/kg (50 mg/kg each of atoltivimab, maftivimab, and odesivimab) for the treatment of reverse transcription-polymerase chain reaction (RT-PCR)-confirmed EBOV infection, was found to be superior in terms of reduction of 28-day mortality compared to ZMapp. This finding from a single, adequate, and well-controlled trial was sufficient to demonstrate clinical efficacy. Nonclinical studies have satisfied the requirements under 21 CFR Section 300.50 and demonstrate that each component contributes to the antiviral activity of the REGN-EB3 combination. Overall, the studies performed in NHPs using REGN-EB3 provided proof-of-concept for REGN-EB3 and were the basis for establishing the dose used in an emergency setting and for clinical trials. However, the mechanisms of action for atoltivimab, maftivimab, and odesivimab have not yet been fully characterized. Two postmarketing requirement (PMRs) and two postmarketing commitments (PMCs) will be issued to further characterize the mechanisms of action for each mAb (Section III.22). Although the PALM trial demonstrated that a single 150 mg/kg dose was efficacious, the Applicant has not provided evidence of an optimally efficacious dose, particularly for patients who present with high viral loads. One concern is that high levels of sGP may compromise the contribution of odesivimab (REGN-3471), and therefore it is unclear whether a higher dose of odesivimab, or of all three mAbs, is needed to fully demonstrate dose optimization. Unfortunately, the clinical trial design needed to assertain the optimal dose may be difficult to conduct in an outbreak setting.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	efficacy endpoint, reduction in 28-day mortality, in the REGN-EB3 arm compared to ZMapp. Mortality in subjects concurrently enrolled in the intention-to-treat (ITT) population was 52/154 (33.8%) in the REGN-EB3 arm and 78/153 (51.0%) in the ZMapp arm with a rate difference of -17.2% (95% confidence interval -28.0% to -4.1%). - Lower efficacy was observed in the PALM RCT in those with a cycle-threshold nucleoprotein gene targets (CtNP) value ≤22 (which correlates with a higher viral load) versus >22. - No experience with treatment for infection by aerosol, needle-stick, (b) (4), was presented in the BLA submission. - The Extension Phase is ongoing following the termination of the initial phase of the PALM trial; however, the results have not been shared with the Applicant and were not included in the interim analysis.	

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and Risk Management	• Infusion-related reactions, including anaphylaxis, are important potential safety concerns for therapeutic proteins, including mAbs, but no conclusive evidence of immunogenicity was found in healthy human volunteers. The assay used in this study, however, did not include validated methods for demonstrating presence of neutralizing antibodies. In the PALM trial, signs or symptoms of anaphylaxis, such as hypotension, were confounded by the underlying EBOV infection. • Pyrexia (or elevation in fever), chills, tachycardia, tachypnea, and vomiting were the most common adverse events (AEs) reported during REGN-EB3 infusion in the PALM trial. • Planned evaluations in the PALM trial were limited to 58 days postinfusion, and limited safety data were collected for subjects in EAP 1846. • Given the mortality associated with EBOV infection and the likelihood that there was a greater risk from untreated infection than from the study medications themselves, pregnant and pediatric subjects, including neonates, were eligible for enrollment into the PALM trial and EAP 1846. – A total of 16 pregnant subjects were enrolled in the PALM trial, the PALM Extension Phase and EAP 1846. – Overall, 77 subjects (20%) of the combined population were in pediatric age groups, with 39 subjects under the age of 18 years enrolled in the PALM trial. Although younger age groups were small, safety findings were consistent across the groups. – Recommended infusion volumes and infusion times varied for different age or weight groups due to endotoxin thresholds associated with the diluent needed for administration. • Limited data have been collected to identify resistance pathways, but no clinical or relevant animal study has fully characterized the potential for clinically significant resistance substitutions associated with one or more of the individual mAbs of REGN-EB3. • No formal vaccine interaction studies were conducted to determine whether REGN-EB3 may inhibit replication of a live vaccine virus indicated for prevention of *Zaire ebolavirus* infection and possibly reduce the efficacy of the vaccine.	Safety data collection was constrained by the challenging circumstances at the clinical study sites, including limited resources and communication with the Applicant, sociopolitical turmoil, and the inherent hazard to health care providers in the setting of an EBOV outbreak. Because the signs and symptoms of EBOV infection are often serious, they confounded the ability to correlate a specific adverse event with REGN-EB3. It is also unclear whether REGN-EB3 had any impact on long-term safety outcomes, such as late recurrence due to persistence in immune-privileged sites (e.g., uveitis or orchitis). Although the assessment of clinical safety experience with REGN-EB3 in the setting of treatment for EBOV infection was limited, the overall safety database was adequate for an evaluation of risk. Having met the primary efficacy objective, superiority in reduction of 28-day mortality, a degree of uncertainty in describing the risk attributable to REGN-EB3 was considered acceptable. The potential and unknown risks to pregnant and pediatric subjects were also considered to be acceptable given the anticipated morbidity and mortality due to EBOV infection.

Conclusions Regarding Benefit-Risk

Zaire ebolavirus (EBOV) infection is a rapidly progressive and often fatal disease. Outbreaks have originated in western and equatorial Africa, some of which have spread internationally. Although a vaccine has recently been approved for prevention of EBOV infection, there are currently no approved antiviral drugs for treatment of EBOV infection, and standard of care is supportive. Antiviral treatments are needed in addition to vaccines when new outbreaks occur, where vaccination programs have not yet been deployed, and cases in which vaccination was not fully protective. REGN-EB3, for the treatment of reverse transcription-polymerase chain reaction (RT-PCR)-confirmed EBOV infection, was found to be superior in terms of reduction of 28-day mortality compared to ZMapp, an investigational control. This finding from a single, adequate, and well-controlled trial was sufficient to demonstrate clinical efficacy and safety and support approval.

Nonclinical studies have demonstrated that each component contributes to the antiviral activity of the REGN-EB3 combination. Overall, the studies performed in nonhuman primate (NHPs) using REGN-EB3 provided proof-of-concept for REGN-EB3 and were the basis for establishing the dose used in an emergency setting and for clinical trials. However, the mechanisms of action for atoltivimab, maftivimab, and odesivimab, and clinically significant resistance substitutions, have not yet been fully characterized. Although the PALM trial demonstrated that a single dose (50 mg/kg each of atoltivimab, maftivimab, and odesivimab) was efficacious, the Applicant has not provided additional evidence of an optimally efficacious dose, particularly for patients who present with high viral loads (CtNP $\leq$22). One concern is that high levels of secreted glycoprotein (sGP) may compromise the contribution of odesivimab (REGN-3471), and therefore it is unclear whether a higher dose of odesivimab, or of all three mAbs, is needed to fully demonstrate dose optimization.

Safety data collection was constrained by the challenging circumstances at the clinical study sites, including limited resources and communication with the Applicant, sociopolitical turmoil, and the inherent hazard to health care providers in the setting of an EBOV outbreak. Because the signs and symptoms of EBOV infection are often serious, they confounded the ability to correlate a specific adverse event with REGN-EB3. It is also unclear whether REGN-EB3 had any impact on long-term safety outcomes, such as late recurrence due to persistence in immune-privileged sites (e.g., uveitis or orchitis). Infusion-related reactions, including anaphylaxis, are important potential safety concerns for therapeutic proteins, including REGN-EB3, but no conclusive evidence of immunogenicity was found in healthy human volunteers.

Although the assessment of clinical experience with REGN-EB3 in the setting of treatment for EBOV infection was limited, the overall demonstration of benefit and risk was adequate to assess REGN-EB3 for the proposed indication. Having met the primary efficacy objective, superiority in reduction of 28-day mortality, a degree of uncertainty in describing the risk attributable to REGN-EB3 was considered acceptable. The potential and unknown risks to pregnant and pediatric subjects were also considered to be acceptable given the anticipated morbidity and mortality due to EBOV infection.

II. Interdisciplinary Assessment

3. Introduction

The Applicant submits this BLA for INMAZEB™ (atoltivimab, maftivimab and odesivimab-ebgn), also known as REGN-EB3, a combination of three human immunoglobulin G (IgG) monoclonal antibodies (mAbs) directed against *Zaire ebolavirus* (EBOV) glycoprotein (GP). The requested indication is for the treatment of infection caused by EBOV in adult and pediatric patients.

Each of three antibodies is a new chemical entity that, in combination, provide antiviral activity by targeting different epitopes of the EBOV GP protein and induction of various antibody-mediated and cell-mediated immune responses. Of note, however, REGN-EB3 has not been evaluated against other species of the *Ebolavirus* or *Marburgvirus* genera.

There are currently no approved antiviral drugs for treatment of EBOV infection. Given the high fatality rates and resulting disruption that occurs with EBOV outbreaks, safe and effective treatments are an important unmet need.

Due to the challenges and limitations associated with studying EBOV infection in the clinical setting, the initial REGN-EB3 development program was based on fulfilling the criteria for approval under the Animal Rule. Nonhuman primate challenge studies provided initial proof-of-concept and informed the choice of dose used in the first treatment studies in EBOV-infected subjects. In collaboration with the World Health Organization (WHO), REGN-EB3 was provided to the emergency response during the 2018 to 2020 outbreak in North Kivu and Ituri provinces in the Democratic Republic of the Congo (DRC). In November 2018, with an extraordinary international and interagency coordination between the US NIAID, the DRC Institut National de Recherche Biomédicale (INRB) and humanitarian nongovernmental organizations (NGOs), the PAmoja TuLinde Maisha (PALM) trial was initiated at four sites. PALM was a multicenter, open-label, randomized controlled superiority trial of four therapeutic candidates, including REGN-EB3. On August 9, 2019, because a superiority finding for REGN-EB3 and mAb114 (ansuvimab) over the active investigational control arm (ZMapp) was demonstrated, the data safety monitoring board (DSMB) recommended stopping PALM before the planned enrollment was complete. PALM was continued with an Extension Phase, where subjects were randomized to either REGN-EB3 or mAb114.

3.1. Approach to the Review

Table 3 provides an overview of the clinical trials to support the benefit and risk assessment for REGN-EB3. The PALM trial (NCT03719586, protocol number 19-I-0003) was the primary source of evidence to support the finding of efficacy. The review of clinical safety considered all available clinical experience in the context of the challenges inherent with EBOV outbreaks and the sociopolitical challenges caused by the location of the outbreak. Because mortality was the primary efficacy endpoint of the PALM RCT, the ascertainment of benefit and the determination of safety may overlap in the setting of an indication with high morbidity and mortality. With the

demonstration of a statistically significant treatment effect on mortality, especially with consistent findings for key secondary efficacy endpoints, a degree of uncertainty with the assessment of safety was acceptable.

Some of these uncertainties that could not be addressed were due to the limited clinical follow-up during the PALM trial and EAP 1846 and whether REGN-EB3 had any impact on long-term outcomes, such as late recurrence due to persistence in immune-privileged sites. Additionally, no formal vaccine interaction studies were performed (see additional discussion in Section II.8.2). Of particular concern is whether REGN-EB3 may inhibit replication of a live vaccine virus indicated for prevention of EBOV infection and possibly reduce the efficacy of the vaccine.

The review team identified five review issues relevant to the evaluation of benefit (Section II.6.4):

- Use of an investigational drug, ZMapp, as an active control versus optimized standard of care (oSOC) alone.
- Lower efficacy in REGN-EB3-treated subjects with high viral loads (baseline CtNP values ≤22) versus subjects with low baseline viral loads (CtNP >22).
- Demonstration of the contribution of each component of the combination
- Adequacy of clinical experience with pediatric subjects.
- Lack of clinical experience with REGN-EB3 for treatment of EBOV infection acquired by routes other than natural transmission.

The review team also identified three review issues relevant to the evaluation of risk and risk management (Section II.7.7):

- The development of resistance against REGN-EB3 has not been adequately characterized.
- Potential risks of immunogenicity.
- Risks associated with the proposed total infusion volumes and infusion times for neonates.

Table 3. Clinical Trials Submitted in Support of Efficacy and/or Safety Determinations[1] for INMAZEB (atoltivimab, maftivimab and odesivimab-ebgn; REGN-EB3)

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized	Number of Centers and Countries
19-I-0003 (PALM trial) (NCT03719586)	Persons with confirmed EBOV infection at a participating ETU	Control type: Active control (ZMapp) Randomization: Randomized Blinding: Open-label Biomarkers: RT-PCR viral load over time	• ZMapp, 50 mg/kg IV q3d ×3 doses, or • REGN-EB3, 150 mg/kg IV ×1 dose, or • mAb114, 50 mg/kg IV ×1 dose, or • remdesivir, IV with a 200 mg loading dose (5 mg/kg for pediatric subjects ≥40 kg) on day 1 followed by 9 to 13 days of once-daily maintenance dosing starting on day 2 and extending through days 10 to 14 Number treated: ZMapp: 152 (153 randomized); REGN-EB3: 154	Primary: 28-day mortality Secondary: • Time to first negative Ebola RT-PCR in blood • Viremia over time • Time to discharge from ETU • Incidence of serious adverse events	Total of 500 subjects initially planned, amended to 725. Actual total enrollment: 681 randomized	Four centers (Beni, Butembo, Katwa, and Mangina), each in 1 country (DRC)
R3470-3471-3479-EBOV-1846 (EAP 1846) (NCT03576690)	Patients with confirmed EBOV infection presenting at an ETU in the DRC	Compassionate-use program Control type: No control Randomization: No randomization Blinding: No blinding	Drug: REGN-EB3 Dose: 150 mg/kg IV x1 dose Number treated: 228 (one died before receiving treatment)	No endpoints were identified	No specific number of individual subjects were planned. Actual enrollment was 229	Seven sites, each in 1 country (DRC)

Source: Reviewer
[1] Includes all submitted clinical trials, even if not reviewed in-depth, except for phase 1 and pharmacokinetic studies.
Abbreviations: DRC, Democratic Republic of the Congo; EBOV, *Zaire ebolavirus*; ETU, Ebola treatment unit; IV, intravenous; PALM, PAmoja TuLinde Maisha; RT-PCR, reverse transcription-polymerase chain reaction

4. Patient Experience Data

Due to the limitations and challenges of conducting a trial for acute EBOV infection (particularly with the social-political environment in the Democratic Republic of the Congo [DRC]), patient experience data were not collected in the PAmoja TuLinde Maisha (PALM) trial. However, for future consideration and to assess long-term outcomes, survivor studies may benefit from the collection of patient experience data. The sequelae of EBOV infection can include arthralgia, myalgia, headache, neuropsychiatric, testicular, and ophthalmic disorders. Survivors of previous outbreaks have also reported varying degrees of functional status (Qureshi et al. 2015). It is unclear whether early intervention with treatments such as REGN-EB3 can mitigate onset of these sequalae.

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
Clinical outcome assessment data submitted in the application		
☐	Patient-reported outcome	
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
Other patient experience data submitted in the application		
☐	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☒	**If no patient experience data were submitted by Applicant, indicate here.**	
Data Considered in the Assessment (but Not Submitted by Applicant)		
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

The pharmacokinetics (PK) of REGN-EB3 was evaluated only in healthy adults. In the PALM trial, the protocol indicated that PK sample collection was not feasible in most settings but stated that samples could be collected where sample processing could be performed safely, and samples stored appropriately. According to the PALM trial report, due to shipment restrictions (see below) and an inability to analyze samples locally, PK data are not available.

The PALM protocol also allowed for collection of optional stored samples. We asked whether these samples could be used for PK analysis. The Applicant contacted the study sponsor, National Institute of Allergy and Infectious Diseases (NIAID), who stated that samples were either not available or would not be shipped from the DRC, and also that equipment availability and lack of staff training precluded analysis of the samples in the DRC (Response to IR submitted 7/9/2020). Additional challenges include the following:

- Unclear whether accurate timing of sample collection relative to dosing was documented for optional stored samples.
- Current duration of stability in human serum is 12 months at -20°C and 24 months at -80°C. It is unclear whether samples would meet these duration and temperature criteria.
- Procedures for decontaminating and shipping infected human serum samples have not been defined and the impact of decontamination on drug concentration has not been evaluated.

Table 5. Summary of General Clinical Pharmacology and Pharmacokinetics

	Drug Information
Characteristic	**Pharmacologic Activity**
Established pharmacologic class (EPC)	REGN-EB3 is a combination of *Zaire ebolavirus* glycoprotein-directed monoclonal antibodies (atoltivimab, maftivimab, and odesivimab).
Mechanism of action	REGN-EB3 is an antiviral drug combination of three recombinant human IgG1κ monoclonal antibodies (atoltivimab, maftivimab, and odesivimab) that inhibit *Zaire ebolavirus*.
Active moieties	The active moieties are atoltivimab, maftivimab, and odesivimab.
QT prolongation	Monoclonal antibodies are unlikely to cause QT prolongation; effects of atoltivimab, maftivimab, and odesivimab on the QT interval were not assessed.

Characteristic	Drug Information Pharmacologic Activity
General Information	
Bioanalysis	Assays measuring atoltivimab, maftivimab and odesivimab in serum of uninfected humans were validated per FDA guidance. PK data are not available in infected humans.
Healthy subjects versus patients	PK data are available in uninfected healthy adults; no PK data are available from infected subjects.
Drug exposure at steady state following the therapeutic dosing regimen (or single dose, if more relevant for the drug)	After a dose of 150 mg/kg (50 mg/kg each antibody) in uninfected adults, the atoltivimab, maftivimab and odesivimab mean C_{max} values were 1,220 mg/L, 1,280 mg/L, and 1,260 mg/L; the mean AUC_{inf} values were 17,100 mg*day/L, 18,700 mg*day/L and 25,600 mg*day/L.
Range of effective dose(s) or exposure	The single efficacy study (PALM) evaluated a dose of 150 mg/kg; exposures were not measured in this study.
Maximally tolerated dose or exposure	
Dose proportionality	At doses of 3 mg/kg (1 mg/kg per antibody) to 150 mg/kg (50 mg/kg per antibody), exposures were dose-proportional.
Bridge between to-be marketed and clinical trial formulations	According to the Applicant, the to-be-marketed formulation is the same as that evaluated in the phase 3 study.
Absorption	
T_{max}	Infusion duration in labeling is 2 to 4 hours, depending on body weight. T_{max} is expected at the end of infusion.
Food effect (fed/fasted) geometric least square mean and 90% CI	Not applicable
Distribution	
Volume of distribution	In uninfected adults, mean atoltivimab, maftivimab, and odesivimab volumes of distribution were 58.2 mL/kg, 57.6 mL/kg, and 56.0 mL/kg.
Elimination	
Mass balance results	Not applicable
Clearance	In uninfected adults, the mean atoltivimab, maftivimab and odesivimab clearance values were 3.08 mL/day/kg, 2.78 mL/day/kg, and 2.02 mL/day/kg.
Half-life	In uninfected adults, the mean atoltivimab, maftivimab and odesivimab half-life values were 21.2 days, 22.3 days, and 25.3 days.
Intrinsic Factors and Specific Populations	
Body weight	PK was only evaluated in healthy adults aged 21 to 60 years and with a body mass index in the normal range. The impact of age (pediatric or geriatric) or obesity has not been evaluated.
Age	
Immunogenicity (for Biologics)	
Bioanalysis	A nonquantitative, titer-based, bridging immunoassay was used to detect anti-atoltivimab, anti-maftivimab, and anti-odesivimab antibodies in uninfected human serum samples.
Incidence Clinical impact	In uninfected humans administered a single dose, no antidrug antibodies were detected.

Abbreviations: AUC_{inf}, AUC from time zero extrapolated to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; PALM, PAmoja TuLinde Maisha; PK, pharmacokinetics; T_{max}, time to C_{max}

5.1. Nonclinical Assessment of Potential Effectiveness

The nonclinical data support the potential effectiveness of REGN-EB3 based on the following findings (see Section III.18 for detailed reviews of these study reports).

Mechanism of Action

- The mechanism of action (MOA) data (Table 6) show that all three monoclonal antibodies (mAbs) contained in the REGN-EB3 cocktail bind with high affinity to recombinant EBOV Makona glycoprotein (GP), with odesivimab (REGN3471) also binding recombinant secreted glycoprotein (sGP).
- Surface plasmon resonance was used to show all three mAbs bind to GP simultaneously and sequentially, and binding is not substantially impacted by lower pH.
- Single-particle, negative-stain electron microscopy (EM) showed the general areas that each mAb binds, and hydrogen–deuterium exchange identified putative epitopes (footprints) for each. Of note, there is significant overlap in the footprints of atoltivimab (REGN3470) and odesivimab (REGN3471).

Table 6. Summary of REGN-EB3 Mechanism of Action Data

mAb	Binding (2 Lots) nM	Binds sGP	Binding GP Sim	Binding GP Seq	Block GP Binding IC_{50} nM	Epitope (Western blot)	Binding Region	Footprint Region[1]
REGN 3470	7.74 and 7.93	No	Yes	Yes	28.5	Linear	Parallel to the viral surface on the outside of the glycan cap	236 to 244: VDNLTYVQL 264 to 287: GKRSNTTGKLIWKV NPEIDTTIGE 298 to 308: TRKIRSEELSF
REGN 3471	8.42 and 8.10	Yes	Yes	Yes	8.74	Linear	Perpendicular to the viral surface within the chalice structure of the trimer at or near the head of the GP in an area that likely overlaps with the glycan cap	114 to 122: KKPDGSECL 139 to 151: HKVSGTGPCAGDF 236 to 244: VDNLTYVQL 265 to 287: KRSNTTGKLIWKVN PEIDTTIGE
REGN 3479	2.97 and 3.70	No	Yes	Yes	10.1	Linear, conforma-tional	Base of GP between GP1/GP2, near the internal fusion loop and cathepsin cleavage site	531 to 545: WIPYFGPAAEGIYT E

Source: DAV analyses
[1] The footprint region encompasses residues that were shielded by the specific mAb in the HDX experiment, indicating a potential interaction with the mAb.
Abbreviations: EC_{50}, half-maximal effective concentration; GP, glycoprotein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; sim, simultaneously; seq, sequentially

- The mechanisms of action have not been completely defined for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479); however, Clinical Virology reached agreement on two postmarketing requirements (PMRs) and two postmarketing commitments (PMCs) with the Applicant (Section III.22) that will address remaining questions regarding MOA.

Cell Culture Antiviral Activity

Cell culture antiviral activity data (Table 7) show that:

- Maftivimab (REGN3479) and REGN-EB3 neutralize four different replication-competent EBOV strains (Kikwit, Makona, Mayinga, and guinea-pig-adapted EBOV Mayinga) in Vero cells with plaque reduction neutralization titers (PRNT)-80 titers of 0.2 to 1.2nM (half-maximal effective concentration (EC_{50}) values were as follows: Kikwit, 0.3nM; Makona, 1.2nM; Mayinga 0.2nM; and guinea-pig-adapted Mayinga, 0.6nM).
- Atoltivimab (REGN3470), maftivimab (REGN3479), and REGN-EB3 were able to neutralize EBOV Makona vesicular stomatitis virus (VSV) pseudotyped virus-like particles (VLPs) with EC_{50} values of 0.27nM, 0.14nM, and 0.41nM, respectively. Odesivimab (REGN3471) had an EC_{50} value >60nM in this assay, which resulted in ~50% neutralization at the highest concentration assessed (100nM).
- Antibody-dependent cellular cytotoxicity (ADCC) signaling through the FcγR3A pathway was characterized for atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 with EC_{50} values of 2.9nM, 1.6nM, and 1.7nM. Maftivimab (REGN3479) signaling was not detected in this assay.
- The binding of C1q to the Fc domain of a mAb is required to initiate complement-dependent cytotoxicity to initiate complement activation, and C1q binding was not detected for any of the REGN-EB3 mAbs.
- A second study showed that atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 induced FcγR3A-mediated antibody-dependent cellular phagocytosis (ADCP) of target cells expressing EBOV Makona GP with EC_{50} values of 578pM, 4.71nM, and 904pM, respectively. Maftivimab (REGN3479) at up to 100nM did not induce FcγR3A-mediated ADCP of target cells.

Table 7. Summary of Antiviral Activity Data for REGBN-EB3

	Live Virus Neut. Titers PRNT-80 (nM)							
Antibody	GP-Adapted	Kikwit	Makona	Mayinga	EBOV VLP EC_{50} Value (nM)	FcγR3A Signaling (ADCC) EC_{50} Value (nM)	FcγR3A-Mediated ADCP EC_{50} Value (nM)	C1q Binding
REGN3470	NA	NA	NA	NA	0.27	2.9	0.58	No
REGN3471	NA	NA	NA	NA	NA	1.6	4.71	No
REGN3479	0.6	0.3	1.2	0.2	0.14	NA	>100	No
REGN-EB3	nd	nd	nd	nd	0.41	1.7	0.9	nd

Source: DAV analyses
Abbreviations: EBOV, *Zaire ebolavirus;* EC_{50}, half-maximal effective concentration; GP-Ad, guinea pig-adapted EBOV Mayinga strain; NA, no activity; nd, not determined; neut, neutralization; PRNT-80, plaque reduction neutralization test; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; VLP, virus-like particle
Guinea Pig Challenge Studies

Guinea Pig Challenge Studies

- EBOV challenge experiments were conducted with guinea pigs lethally challenged by intraperitoneal (IP) injection with guinea-pig-adapted EBOV Mayinga (EC_{50} value in Vero cells 0.6nM) and treated IP with the individual REGN-EB3 mAbs 1 day after challenge or combinations of REGN-EB3 mAbs 3 days after challenge.
- Guinea pigs challenged with guinea-pig-adapted EBOV Mayinga and treated with maftivimab (REGN3479) 1 day after challenge had the greatest 28-day survival rate (4/6, 67%; Table 8).

Table 8. Summary of Data From Guinea Pig Lethal Challenge Studies

Group	Size	Treatment	Dose	Survivors (%)	MTD (n)	Day 4 Mean Titer (PFU/mL) (n)
RG1	6	REGN3470, 1 dpi	5 mg	0 (0)	11 (6)	8.10E +02 (1)
RG2	6	REGN3471, 1 dpi	5 mg	2 (33)	9.75 (4)	3.30E +03 (1)
RG3	6	Placebo, 1 dpi	none	0 (0)	6.5 (6)	1.74E +06 (5)
RG4	6	REGN3474, 1 dpi	5 mg	0 (0)	7.5 (6)	5.66E +05 (3)
RG5	6	REGN3479, 1 dpi	5 mg	4 (67)	6.5 (2)	3.00E +06 (1)
RG6	**6**	**REGN-EB3, 3 dpi**	**5 mg**	**2 (33)**	**6.33 (4)**	**7.97E +04 (3)**
RG7	6	REGN3470-REGN3471-REGN3051, 3 dpi	5 mg	2 (33)	7.67 (4)	8.05E +02 (4)
RG8	6	REGN3051 (Placebo), 3 dpi	none	0	5 (6)	3.40E +06 (6)
RG9	6	REGN3470-REGN3479-REGN3051, 3 dpi	5 mg	1 (17)	7 (5)	5.06E +05 (2)
RG10	6	REGN3471-REGN3479-REGN3051, 3 dpi	5 mg	2 (33)	6 (4)	2.84E +06 (3)

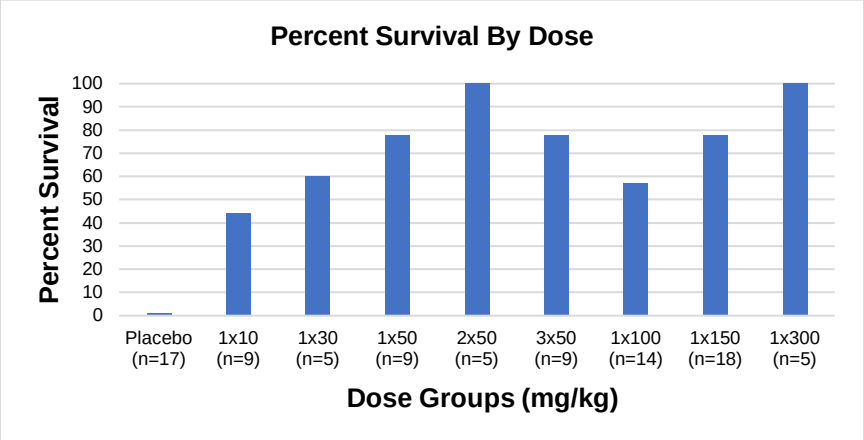
Source: DAV analysis
Abbreviations: dpi, day postinfection; MTD, maximum tolerated dose; PFU, plaque-forming unit; REGN3051, placebo; REGN3474, fourth mAb under consideration but not selected for the REGN-EB3 cocktail; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab
The limited activity of atoltivimab (REGN3470) and odesivimab (REGN3471) raised concerns about breadth of activity early in the development program, but these concerns were mitigated by additional nonclinical data.

- The limited activity of atoltivimab (REGN3470) and odesivimab (REGN3471) raised concerns about breadth of activity early in the development program, but these concerns were mitigated by additional nonclinical data.
- Concerns about polymorphisms and adaptive changes in GP were mitigated by neutralization data for several EBOV strains that were similar (Table 7), comparable activity as other mAb cocktails in nonhuman primate (NHP) studies (Section III.18), and efficacy in human clinical trials.
- This was the only experiment that evaluated the activity of the individual and various combinations of three REGN-EB3 mAbs. Given the limitations of this model and the experimental approach, it is not clear to what extent each mAb contributes to the overall antiviral activity or if each mAb is required for the cocktail to be effective. The cocktail formulations used in this experiment dosed each mAb at one-third the concentration used for the single mAb assessments.
- Clinical Virology reached agreement on two PMRs and two PMCs with the Applicant (Section III.22) that will address remaining questions regarding the MOA of each mAb and the potential overlap in activity.

Nonhuman Primate EBOV Lethal Challenge Studies

Rhesus macaques challenged with a lethal dose of EBOV and treated with REGN-EB3 at all doses assessed, survived at a higher rate than animals that received placebo (Figure 1). NHPs (n=74) were challenged with EBOV Kikwit and treated with REGN-EB3 at various doses, with a dose-dependent response being observed from 10 to 50 mg/kg (Figure 1).

Figure 1. Mean Percentage Survival Across the REGN-EB3 Development Program

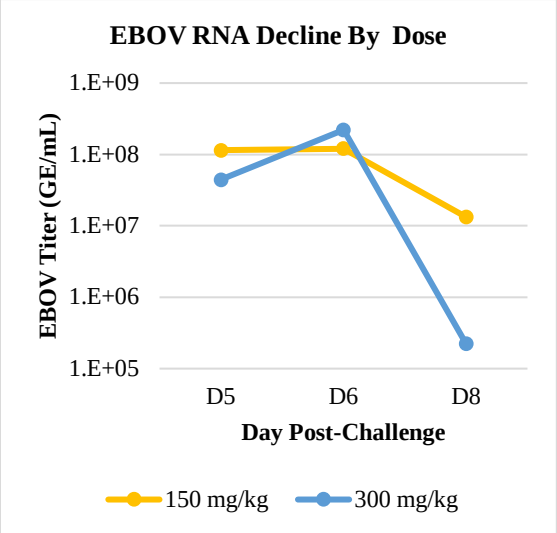


Source: DAV analysis
Abbreviations: n, number of subjects in subgroup

At doses >50 mg/kg, variability within the NHP model and across study sites made it difficult to discern any real difference. For example, in study (b) (4) 2018-008 R3479-PM-18140, five NHPs were challenged intramuscularly (IM) with 1,000 PFU of EBOV on Day 0 and treated with 1×100 mg/kg administered intravenously on Day 5; only one NHP survived (20%) to ≥47 days postchallenge. However, in studies AP-14-017 IX and X, 4/4 (100%) and 4/5 (80%) NHPs survived under the same experimental parameters, but the studies were conducted at different research institutes (Section III.18) and survival was assessed at ≥28 days postchallenge.

In study (b) (4) 2018-008 R3479-PM-18140, a comparison of serum EBOV RNA decline from Day 5 (before initiation of treatment) to Day 8 showed a mean 1.2 $\log_{10}$ GE/mL decrease for the 150 mg/kg REGN-EB3 cohort (n=4 macaques) and a 2.1 $\log_{10}$ GE/mL decline for the 300 mg/kg REGN-EB3 (n=5 macaques) treatment cohort (see Clinical Virology appendix, Section 2.4 for complete details) (Figure 2). Therefore, the 300 mg/kg dose may lead to a steeper and more rapid decline in EBOV RNA (GE/mL).

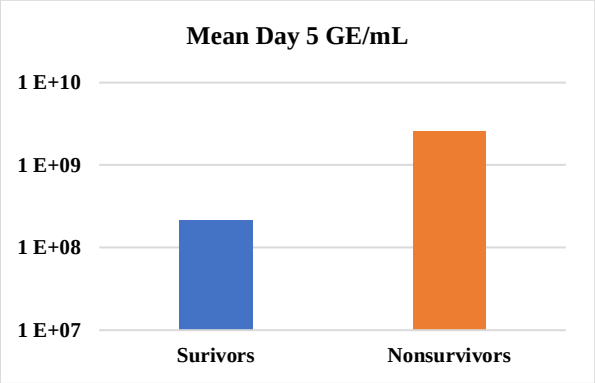
Figure 2. EBOV RNA Decline by Dose Comparing Single Doses of 150 mg/kg vs. 300 mg/kg



Source: DAV analysis
Abbreviations: EBOV, *Zaire ebolavirus*, RNA, ribonucleic acid

NHPs were treated with a single dose of 150 mg/kg REGN-EB3 administered 5 days after challenge; 14/18 (77.8%) survived as a result of treatment (Figure 3). For the 14 NHPs that survived, the mean Day 5 titer was 2.16×10^8 GE/mL, compared to a mean Day 5 titer of 2.54×10^9 GE/mL for nonsurvivors (Figure 3).

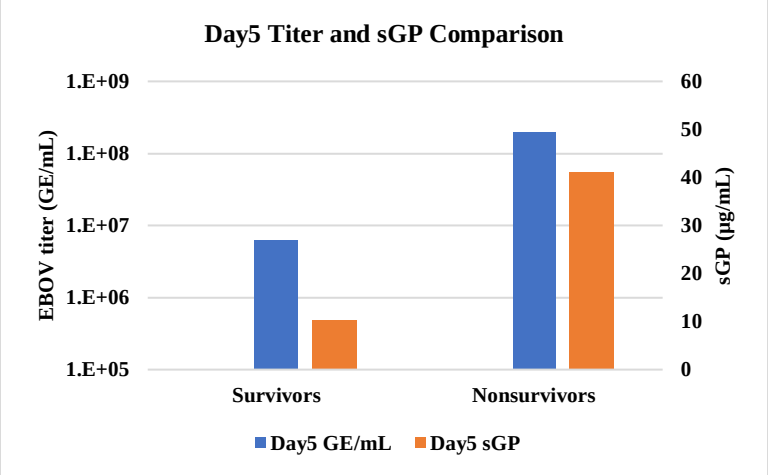
Figure 3. Mean EBOV Titer at Day 5 Prior to Treatment Among NHPs Treated With 150 mg/kg REGN-EB3



Source: DAV analysis
Y-axis is GE/mL. Survivors, n=14; nonsurvivors, n=4
Abbreviations: EBOV, *Zaire ebolavirus*, GE, genome equivalent; NHP, nonhuman primate

Secreted GP concentrations were determined for nine NHPs treated with 150 mg/kg, six and three of which survived and died, respectively, following EBOV challenge. For the survivors, the mean Day 5 titer and sGP concentration were 6.2×10^6 GE/mL and 10.3 µg/mL, respectively, compared to 1.8×10^8 GE/mL and 41.3 µg/mL, respectively, for the nonsurvivors (Figure 4).

Figure 4. Mean Day 5 Titers and sGP Concentrations for NHPs that Received 150 mg/kg of REGN-EB3



Source: DAV analysis
Abbreviations: EBOV, *Zaire ebolavirus*, GE, genome equivalent; NHP, nonhuman primate; sGP, secreted glycoprotein

These results indicate that a higher dose may be more effective for NHPs with baseline EBOV titers $>1\times10^8$ GE/mL and with sGP concentrations >10.3 µg/mL (Section II.6.4.2).

One study tested a dose of 300 mg/kg in NHPs 5 days after challenge. All survived, but only one of the five NHPs had a Day 5 titer of 1.6×10^8 GE/mL and an sGP concentration of 13.6 µg/mL. Variability in the NHP model hampers assessment of higher doses.

Overall, the studies performed in NHPs using REGN-EB3 provided proof-of-concept that REGN-EB3 has antiviral activity and was the basis for establishing the 150 mg/kg dose used in clinical trials in an emergency setting.

6. Evidence of Benefit (Assessment of Efficacy)

6.1. Assessment of Dose and Potential Effectiveness

A single dose of 150 mg/kg (50 mg/kg each for atoltivimab, maftivimab, and odesivimab) was evaluated in the PALM trial.

The human dosing regimen (a single dose of 150 mg/kg) was based on results from lethal challenge studies in an exploratory NHP model (Section II.5.1). The predicted fully effective dose in NHPs is a single dose of 100 mg/kg. However, note that the NHP model is not sufficiently characterized and the studies were not powered for efficacy (Section II.6.4.2).

We compared exposures in NHPs and humans to assess whether human exposures were expected to be similar to or exceed those associated with the effective dose in NHPs. Due to the sparsity of the data and the lack of validated assays for measurement of REGN-EB3 in infected NHPs, conclusions could not be drawn from the PK data in infected NHPs. Relative to uninfected NHPs administered 150 mg/kg, 150 mg/kg administered to uninfected humans resulted in higher exposures. Assuming a similar impact of infection on PK in NHPs and humans, the PK data in uninfected NHPs and humans provide support for the single 150 mg/kg dose evaluated in the PALM trial.

The dose selected for the PALM trial was reasonable and was demonstrated to be effective in comparison to the active control. A higher dose of REGN-EB3 may provide additional benefit to patients infected with EBOV and with high baseline viral loads (Section II.6.4.2). Of note, PK in infected humans was not evaluated in the PALM trial, precluding evaluation of exposure-response relationships for efficacy or safety.

6.2. Design of Clinical Trials Intended to Demonstrate Benefit to Patients

6.2.1. Trial Design

The PALM trial was designed as a master protocol and serves as the primary basis for the efficacy assessment. There are several advantages for implementing a master protocol design, including the ability to: 1) allow for the evaluation of multiple investigational treatments compared to a shared active investigational control arm, 2) add or remove arms, 3) reduce the sample size, and 4) use a common infrastructure with consistent data collection. While there is no consensus across the statistical community, many suggest that a master protocol does not require adjustment for multiple comparisons among treatment groups (Woodcock and LaVange 2017) because each comparison can be considered a separate trial. In the PALM trial, the three

investigational treatment arms belonged to different sponsors. Each sponsor did not have multiple chances to "win," therefore no multiplicity adjustment was deemed necessary.

The trial began with the evaluation of two investigational treatment arms compared to a shared active investigational control arm (ZMapp), and subjects were randomized at a 1:1:1 ratio (refer to Section II.6.4.1). On January 26, 2019, REGN-EB3 was added as the fourth investigational treatment arm, and subjects were subsequently randomized at a 1:1:1:1 ratio. Although the trial was open-label, the trial sponsor (NIAID) incorporated two randomization block sizes to prevent clinicians charged with administering the study drugs from guessing which drug would be administered, thus reducing the potential for selection bias by staff members at Ebola treatment units.

The trial initially targeted 125 subjects per arm based on an expected 28-day mortality rate of 30% in the ZMapp group, with a 50% relative reduction in the experimental treatment. The expected mortality rate of 30% in the ZMapp + oSOC control arm was based, in part, on a meta-analysis of eight clinical studies conducted during the 2014 to 2016 West African Ebola outbreak. This meta-analysis indicated that mortality rates within PREVAIL II (NCT02363322, a randomized controlled trial designed to assess the efficacy of ZMapp), were lower than in other studies across both the treatment and control arms. Hence, the expected mortality rate with ZMapp in the PALM trial could be anticipated to be higher than the point estimate from PREVAIL II. On July 17, 2019, an amendment was submitted (b) (4) requesting enlargement of the sample size to 725 to increase power and allow for detection of a smaller, but clinically meaningful, treatment effect than the original assumed 50% decrease in mortality rate.

On August 9, 2019, the data safety monitoring board (DSMB) recommended stopping PALM before the planned enrollment was met and also recommended the Extension Phase commence with only REGN-EB3 and mAb114 (ansuvimab) because a superiority finding for REGN-EB3 and mAb114 over the active investigational control arm (ZMapp) was demonstrated. As a result, only 684 subjects were enrolled; they form the basis of the efficacy assessment.

The primary efficacy endpoint was the 28-day mortality rate. Additional design information is available in Section III.15.

6.2.2. Eligibility Criteria

Males or females of any age with documented positive reverse transcription-polymerase chain reaction (RT-PCR) (Cepheid assay) for acute EBOV infection within 3 days prior to enrollment and who had symptoms of any duration were eligible for the trial. Neonates (defined as ≤7 days old) born to a mother who was RT-PCR–positive for acute EBOV were presumed to be RT-PCR–positive for acute EBOV at delivery and were eligible for enrollment even prior to RT-PCR confirmation (i.e., obtaining those results could lead to unnecessary delay). Subjects must have agreed not to enroll in another study of an investigational agent prior to completion of Day 28 of the study.

Subjects who had prior treatment with any investigational antiviral drug therapy against EBOV infection within five half-lives or 30 days, whichever was longer, prior to enrollment were not eligible to enroll in the study. Prior vaccination for prevention of EBOV was permitted.

See Section III.15 for key inclusion and exclusion criteria.

6.2.3. Statistical Analysis Plan

The primary efficacy analysis compared REGN-EB3 to ZMapp using Boschloo's exact test. The analysis was conducted on the intention-to-treat (ITT) concurrent population. This population included all randomized subjects when REGN-EB3 and ZMapp were concurrent treatment arms. The subjects were analyzed based on the randomized treatment.

As stated in the protocol, most clinical trials that intend to provide definitive evidence of efficacy ensure strict control of the two-sided type 1 error rate at an alpha level of 0.05, with adjustments for multiple comparisons of arms. This necessitates large sample sizes to ensure high power. The circumstances of high mortality, intermittent and small outbreaks, along with the need to identify effective treatments as quickly as possible potentially justify less austere statistical penalties. As a result, the primary analysis comparing each investigational treatment arm to the shared investigational control arm used a two-sided alpha of 0.05 allocated over interim and final analyses.

At the fourth interim analysis, the trial results crossed the prespecified efficacy boundary. At the time of the interim analysis, 499 participants were enrolled with at least 10 days of follow-up. The 10-day mortality rate was utilized because it was similar to the 28-day mortality rate. This was verified by the final analysis results, which showed that most subjects died on or before the first 11 days.

The final analysis occurred at the fifth interim analysis, and the corresponding interim monitoring boundary was used to assess significance. Thus, a p-value of <0.028 (two-sided) for the comparison of REGN-EB3 to ZMapp was required to claim statistical significance for the primary endpoint.

For more details, please refer to Section III.15.

6.3. Results of Analyses of Clinical Trials/Studies Intended to Demonstrate Benefit to Patients

This section summarizes the subject disposition, baseline demographics, clinical characteristics, and primary and key secondary efficacy results to support the efficacy of REGN-EB3 in reducing 28-day mortality over ZMapp in subjects with confirmed *Zaire ebolavirus* disease (EVD).

6.3.1. Disposition, Baseline Demographics, and Baseline Clinical Characteristics

Disposition

In the PALM trial, 684 subjects were enrolled, and three enrolled subjects died before randomization. In total, there were 681 subjects randomized. Among randomized subjects, 159 subjects were randomized to the REGN-EB3 arm and 169 were randomized to the ZMapp arm. These 328 subjects comprise the ITT overall population. Twenty-one subjects were excluded from the ITT overall population, resulting in 307 subjects in the primary efficacy analysis population, referred to as the ITT concurrent analysis population. The reasons for exclusion of the 21 subjects were as follows: 15 subjects were randomized to ZMapp prior to REGN-EB3 being added to the trial (this is referred to as the ITT Amendment 3 analysis population and

includes 313 subjects), and the remaining 6 subjects received another treatment when either REGN-EB3 or ZMapp was not available. Five subjects received REGN-EB3 when ZMapp was not available, and one subject received ZMapp when REGN-EB3 was not available. Additional details are provided in Section III.16.2.

Table 9. Subject Screening and Randomization, PALM Trial

Analysis Population	REGN-EB3	ZMapp	Total
Subjects died before randomization	–	–	3
Randomized (ITT overall)	159	169	328
Randomized before Amd 3 (1/26/2019)	–	15	15
Randomized after Amd 3 (ITT Amd 3)	159	154	313
Randomized but died before receiving study drug	5	1	6
Subjects randomized during a drug shortage of either REGN-EB3 or ZMapp	5	1	6
Randomized and treated (APT)	154	153	307
ITT Concurrent analysis population	154	153	307
Safety analysis population	154	168	322

Source: Statistical reviewer, ADSL
Abbreviations: APT, all patients treated; ITT, intention-to-treat; ITT Amd 3, ITT Amendment 3 Analysis Set; PALM, PAmoja TuLinde Maisha

Subject-disposition information for the ITT population following Amendment 3 (ITT Amd 3) is summarized in Table 10. By Day 28, 52 (32.7%) subjects randomized to REGN-EB3 had died, and 79 (51.3%) subjects randomized to ZMapp had died. One subject in each arm died after Day 28 but before Day 58. The percentage of subjects completing Day 58 was 66.7% in the REGN-EB3 arm and 48.1% in the ZMapp arm.

Table 10. Disposition, ITT Amendment 3 Population, PALM Trial

Disposition (ITT Amd 3)	REGN-EB3	ZMapp	Total
All randomized after Amd 03 (ITT Amd 3)	159	154	313
Positive baseline CtNP	159	153*	312
Negative baseline CtNP	0	0	0
Subjects completed Day 28 visit	107 (67.3%)	75 (48.75)	182
Subjects died before Day 28	52 (32.7%)	79 (51.3%)	131
Subjects completed Day 58 visit	106 (66.7%)	74 (48.1%)	180
Subjects died before Day 58	53 (33.3%)	80 (51.9%)	133

Source: Statistical reviewer, ADSL
* One subject in the ZMapp arm did not have baseline CtNP measurement.
Abbreviation: Amd 3, ITT Amendment 3 Analysis population; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; N, number of subjects; n, number of subjects with at least one event; PALM, PAmoja TuLinde Maisha
Baseline Demographics and Clinical Characteristics

Baseline Demographics and Clinical Characteristics

The subjects' demographic characteristics were similar in the two arms. Overall, slightly more female subjects (55.4%) were enrolled compared to male subjects (44.6%); the median age was 28 years, with a range of 1 day to 73 years. Most subjects (84.1%) were enrolled at the Beni and Butembo sites. The other two sites, Katwa and Mangina, enrolled only 16% of the subjects. A baseline CtNP >22 (low viral load) was observed in 57.5% of subjects, 24.4% of subjects self-reported having received vaccination (a recombinant vesicular stomatitis virus expressing the EBOV glycoprotein, or rVSV-ZEBOV) prior to baseline, and 10.5% of subjects were malaria positive at baseline. The overall median number of days from symptom onset to randomization was 5 days.

Table 11. Baseline Demographic and Clinical Characteristics, ITT Concurrent Population, PALM Trial

Characteristics	REGN-EB3 (N=154)	ZMapp (N=153)	Total (N=307)
Sex			
Female	90 (58.4%)	80 (52.3%)	170 (55.4%)
Male	64 (41.6%)	73 (47.7%)	137 (44.6%)
Age (years)[a]			
Mean (SE)	28.4 (1.5)	30.5 (1.3)	29.4 (1.0)
Median	26.0	30.0	28.0
Range	(0.04, 73.0)	(0.00, 70.0)	(0.00, 73.0)
SD	18.2	16.5	17.4
Age category 1			
0 ≤ age < 1 month	1 (0.6%)	2 (1.3%)	3 (1.0%)
1 month ≤ age < 1 year	4 (2.6%)	1 (0.7%)	5 (1.6%)
1 year ≤ age < 6 years	18 (11.7%)	13 (8.5%)	31 (10.1%)
6 years ≤ age < 12 years	8 (5.2%)	4 (2.6%)	12 (3.9%)
12 years ≤ age < 18 years	8 (5.2%)	8(5.2%)	16 (5.2%)
18 years ≤ age < 50 years	93 (60.4%)	105 (68.6%)	198 (64.5%)
50 years ≤ age < 65 years	17 (11.0%)	18 (11.8%)	35 (11.4%)
Age ≥ 65 years	5 (3.2%)	2 (1.3%)	7 (2.3%)
Age category 2			
Age < 18 years	39 (25.3%)	28 (18.3%)	67 (21.8%)
Age ≥ 18 years	115 (74.7%)	125 (81.7%)	240 (78.2%)
Site			
Beni	67 (43.5%)	68 (44.4%)	135 (44.0%)
Butembo	63 (40.9%)	60 (39.2%)	123 (40.1%)
Katwa	10 (6.5%)	12 (7.8%)	22 (7.2%)
Mangina	14 (9.1%)	13 (8.5%)	27 (8.8%)
CtNP (≤22, >22)			
≤22	66 (42.9%)	64 (42.1%)	130 (42.5%)
>22	88 (57.1%)	88 (57.9%)	176 (57.5%)
Reported rVSV-ZEBOV vaccination			
Yes	34 (22.1%)	41 (26.8%)	75 (24.4%)
No	118 (76.6%)	112 (73.2%)	230 (74.9%)
Unknown	2 (1.3%)	0(0%)	2 (0.7%)
Pregnancy test			
n	90	80	170
Positive	2 (2.2%)	4 (5.0%)	6 (3.5%)
Negative	65 (72.2%)	57 (71.3%)	122 (71.8%)
Reason unknown	4 (4.4%)	0 (0%)	4 (2.4%)
Not applicable	19 (21.1%)	19 (23.8%)	38 (22.4%)
Malaria status			
Positive	17 (12.4%)	12 (8.6%)	29 (10.5%)
Negative	120 (87.6%)	127 (91.4%)	247 (89.5%)
Days from symptom onset to randomization			
n	154	152	306
Mean (SE)	5.4 (0.3)	5.5 (0.3)	5.4 (0.2)
Median	5.0	5.0	5.0
Range	(1, 17)	(1, 21)	(1, 21)
SD	3.2	3.6	3.4

Characteristics	REGN-EB3 (N=154)	ZMapp (N=153)	Total (N=307)
Baseline weight (kg)			
n	154	153	307
Mean (SE)	46.3 (1.6)	49.6 (1.5)	47.9 (1.1)
Median	50.0	52.0	51.0
Range	(2.4, 90.0)	(2.0, 100.0)	(2.0, 100.0)
SD	20.2	18.8	19.6
Baseline CtNP ≤22			
n	66	64	130
Mean (SE)	19.2 (0.2)	18.5 (0.2)	18.9 (0.2)
Median	19.8	18.3	18.9
Range	(13.7, 22.0)	(14.8, 21.9)	(13.7, 22.0)
SD	1.9	1.7	1.8
Baseline CtNP >22			
n	88	88	176
Mean (SE)	27.8 (0.4)	26.7 (0.4)	27.3 (0.3)
Median	27.6	26.1	26.4
Range	(22.1, 36.4)	(22.2, 37.0)	(22.1, 37.0)
SD	4.0	3.8	3.9
Baseline CtNP			
n	154	152	306
Mean (SE)	24.11 (0.43)	23.26 (0.42)	23.69 (0.28)
Median (Q1, Q3)	22.65 (20.1, 28.1)	22.85 (18.8, 26.4)	22.80 (19.5,26.9)
Range	(13.7, 36.4)	(14.8, 37.0)	(13.7, 37.0)
SD	5.35	5.12	5.25
Baseline CtGP			
n	154	152	306
Mean (SE)	28.8 (0.4)	28.1 (0.4)	28.5 (0.3)
Median	27.8	27.9	27.8
Range	(19.5, 44.1)	(19.9, 45.0)	(19.5, 45.0)
SD	5.0	4.8753	4.9
Baseline ALT			
Mean (SE)	338.7 (39.16)	374.7 (38.39)	356.2 (27.41)
Median (Q1, Q3)	165.0 (56, 418)	223.5 (47, 564)	190.0 (52, 537)
Range	(13, 2000)	(5, 2000)	(5, 2000)
SD	441.3	420.5	430.8
Baseline AST			
Mean (SE)	608.7 (72.67)	712.3 (77.38)	657.8 (52.97)
Median (Q1, Q3)	225.5 (98, 941)	351.0 (109, 1404)	278.0 (98, 1112)
Range	(31, 2000)	(29, 2000)	(29, 2000)
SD	697.0	704.9	700.7
Baseline creatinine			
Mean (SE)	2.34 (0.236)	2.64 (0.279)	2.48 (0.182)
Median (Q1, Q3)	1.00 (0.7, 4.0)	1.10 (0.7, 3.2)	1.05 (0.7, 3.8)
Range	(0.20, 12.50)	(0.30, 14.30)	(0.20, 14.30)
SD	2.644	3.014	2.827

Source: Statistical reviewer, ADSL and SAS software used
Abbreviations: N, number of subjects in treatment group; n, number of subjects with given characteristic; SE, standard error; SD, standard deviation
[a] Most subjects only have year reported for their birthdate
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CtGP, cycle-threshold glycoprotein gene targets; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; N, number of subjects in treatment group; n, number of subjects with given characteristic; PALM, PAmoja TuLinde Maisha; rVSV, vesicular stomatitis virus; SE, standard error; Std: standard deviation.

6.3.2. Primary and Key Secondary Efficacy Results

Primary Efficacy Endpoint

The Applicant's primary efficacy results were confirmed by the statistical review team and demonstrated superiority of REGN-EB3 compared to ZMapp in reducing the 28-day mortality rate in subjects with confirmed EVD (Table 12). The difference in 28-day mortality rate between REGN-EB3 and ZMapp was -17.2% (95% CI -28.0, -4.1).

Of note, the 95% CI and Boschloo's two-sided p-value generated by the reviewer were slightly different from those of the Applicant due to the software used for the analyses. The Applicant used R and the statistical reviewer used Statistical Analysis System (SAS). The differences are negligible and did not change the conclusion of the trial. The Applicant's results were used in the label.

Table 12. Summary of 28-Day Mortality in the Primary Efficacy Analysis, ITT Concurrent Population, PALM Trial*

Population	REGN-EB3 (N=154) Death/Total (%)	ZMapp (N=153) Death/Total (%)	Rate Difference % (95% CI)[a]	Boschloo's Two-Sided P-Value[b]
ITT Concurrent	52/154 (33.8%)	78/153 (51.0%)	-17.2 (-28.0, -4.1)	0.0023

Source: Statistical reviewer, ADSL and SAS software were used.
[a] The exact confidence interval was based on inverting two one-sided tests in StatXact.
[b] P-value based on Boschloo's test with a default gamma of 0 in StatXact.
* The 95% CI and two-sided P-value are slightly different from the Applicant's due to the software used for the analysis; the differences do not affect the conclusion of the trial. The Applicant used R and the statistical reviewer used SAS. The Applicant's results were used in the label.
Abbreviations: CI, confidence interval; ITT, intention-to-treat; N, number of subjects; PALM, PAmoja TuLinde Maisha;
Sensitivity Analyses of the Primary Efficacy Endpoint

Sensitivity Analyses of the Primary Efficacy Endpoint

The primary efficacy endpoint results were consistent across different analysis populations (Table 13). The analysis populations were the Overall ITT, ITT Amendment 3, All Patients Treated (APT), and two other populations modified from the ITT Concurrent population.

Table 13. Summary of 28-Day Mortality in Different Analysis Populations, PALM Trial

Population	REGN-EB3 Death/Total (%)	ZMapp Death/Total (%)	Rate Difference % (95% CI)[a]	Boschloo's Two-Sided P-Value[b]
ITT Concurrent	52/154 (33.8%)	78/153 (51.0%)	-17.2 (-28.0, -4.1)	0.0023
ITT Overall	52/159 (32.7%)	84/169 (49.7%)	-17.0 (-27.5, -4.6)	0.0020
APT (All treated)	47/154 (30.5%)	78/153 (51.0%)	-20.5 (-31.1, -8.1)	0.0003
ITT Amd 3	52/159 (32.7%)	79/154 (51.3%)	-18.6 (-29.3, -6.1)	0.0008
ITTC Sensitivity 1[c]	52/153 (34.0%)	79/153 (51.6%)	-17.7 (-28.5, -5.1)	0.0019
ITTC Sensitivity 2[d]	52/154 (33.8%)	78/147 (53.6%)	-19.3 (-30.4, -6.6)	0.0006

Source: Statistical reviewer, ADSL and SAS software were used
[a] The exact confidence interval was based on inverting two one-sided tests in StatXact.
[b] P-value based on Boschloo's test with a default gamma of 0 in StatXact.
[c] One subject who was false positive was excluded from the ITT concurrent population.
[d] Six subjects who received ZMapp and were rerandomized to receive either REGN-EB3 or mAb114 after the trial was stopped.
Abbreviations: APT, all patients treated; CI, confidence interval; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha;

Secondary Efficacy Endpoints

Mortality distributed by study day was a key secondary endpoint. Most deaths, 36/53 (67.9%) in the REGN-EB3 arm and 51/79 (64.6%) in the ZMapp arm, occurred within the first 3 days of the trial (Table 14). With three exceptions, all deaths occurred within the first 11 days. One death in

the ZMapp arm occurred on Day 18. From Day 28 to Day 58, there were two deaths, one in each arm.

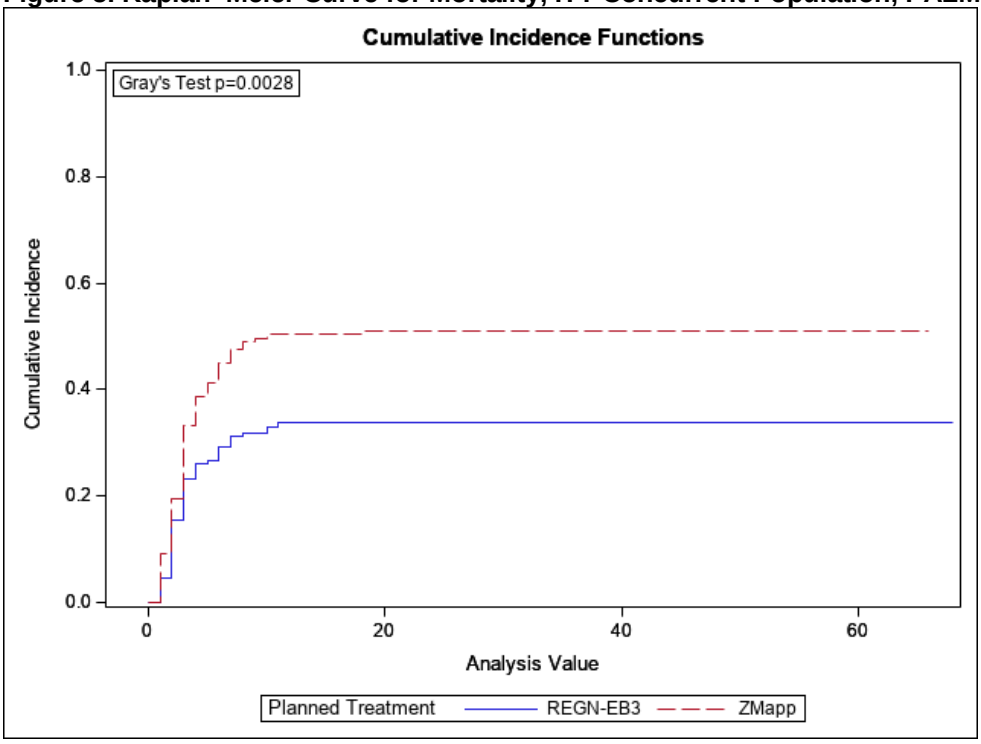
Table 14. Number of Deaths by Study Day, ITT Concurrent population, PALM Trial

Parameter	REGN-EB3 (N=154)	ZMapp (N=153)
Total number of subjects who died, n (%)	53 (34.4)	79 (51.6)
Study day of death, n (%)		
Day 1	7 (4.5)	14 (9.2)
Day 2	17 (11.0)	16 (10.5)
Day 3	12 (7.8)	21 (13.7)
Day 4	4 (2.6)	8 (5.2)
Day 5	1 (0.6)	4 (2.6)
Day 6	4 (2.6)	6 (3.9)
Day 7	3 (1.9)	4 (2.6)
Day 8	1 (0.6)	2 (1.3)
Day 9	0	1 (0.7)
Day 10	2 (1.3)	1 (0.7)
Day 11	1 (0.6)	0
Days 12 to 17	0	0
Day 18	0	1 (0.7)
Days 19 to 27	0	0
Day 28	0	0
Days 29 to 35	0	0
Day >35	1 (0.6)	1 (0.7)

Source: Statistical reviewer, ADSL and SAS software were used.
Abbreviations: ITT, intention-to-treat; N, number of subjects; n, number of subjects in subgroup; PALM, PAmoja TuLinde Maisha,

The Kaplan–Meier (KM) curve for the cumulative incidence of death is shown in Figure 5. Because most deaths occurred within the first 3 days, the cumulative incidence of death increased sharply in the first few days in both arms. After Day 3, the cumulative incidence of death in the REGN-EB3 arm remained lower than that in the ZMapp arm. The log-rank test indicated a significant difference in the curves over time (p=0.0028).

Figure 5. Kaplan–Meier Curve for Mortality, ITT Concurrent Population, PALM Trial



Source: Statistical reviewer, ADTTE and SAS software were used.
Abbreviations: ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha

6.3.3. Subgroup Analyses for the Primary Efficacy Endpoint

Analyses were conducted to assess the treatment effect for subgroups defined by various demographic and clinical characteristics at baseline. The treatment effect of REGN-EB3 compared to the ZMapp appeared consistent across most baseline subgroups of age, gender, site, days from symptom onset to randomization, and other baseline factors analyzed. See Section III.16 for details.

For alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine, the higher the baseline values over the upper limit of normal (ULN), the higher the 28-day mortality rate in both arms. In addition, the 28-day mortality rates in the REGN-EB3 arm were lower than those in the ZMapp arm across these subgroups. The impact of baseline viral load is discussed in Section II.6.4.2.

Of note, the sample sizes for many subgroups were small, which limits the ability to detect trends with certainty. Numerous subgroup analyses were conducted without any adjustment for the multiple analyses, which could result in spurious findings due to chance.

6.4. Review Issues Relevant to the Evaluation of Benefit

The review team concluded that the results of the PALM trial support the proposed indication. The review team did not identify any issues with assessing superiority of REGN-EB3 over

ZMapp for the primary efficacy endpoint (28-day mortality); therefore, no further discussion is warranted in this subsection.

The review issues relevant to the evaluation of benefit focus on:

- Use of an investigational drug, ZMapp, as an active control versus oSOC alone
- Lower efficacy in REGN-EB3-treated subjects with high viral loads (baseline CtNP values ≤22) versus subjects with low baseline viral loads (CtNP >22)
- Demonstration of the contribution of each component of the combination
- Adequacy of clinical experience with pediatric subjects and inclusion of labeled recommendations for low-birth-weight neonates born to EBOV-infected mothers
- Lack of clinical experience with REGN-EB3 for treatment of EBOV infection acquired by routes other than natural transmission

6.4.1. Use of an Investigational Drug, ZMapp, as an Active Control Versus Optimized Standard of Care Alone

Issue

Use of an investigational drug, ZMapp, as an active control versus oSOC alone raised concerns about the interpretability of results in the PALM trial.

Background

ZMapp was previously investigated in the PREVAIL II trial but has not been approved in any country for the treatment of EBOV infection. During the development of the PALM trial, the protocol allowed for country-specific preferences about what constitutes an ethical and scientifically acceptable control arm. Given the state of equipoise for ZMapp, the master protocol contained two options for the control arm as suggested by the World Health Organization (WHO) Research and Development Ebola Therapeutics Committee: either ZMapp + oSOC or oSOC alone. The two options for the control arm resulted in two possible trial designs:

- Option 1: ZMapp as the control arm (four arms, ZMapp + oSOC vs. Drug A + oSOC vs. Drug B + oSOC vs. Drug C + oSOC)
- Option 2: oSOC alone as the control arm (five arms, oSOC vs. ZMapp + oSOC vs. Drug A + oSOC vs. Drug B + oSOC vs. Drug C + oSOC)

The decision about the appropriate control arm was at the discretion of the host country. The PALM trial initially enrolled participants only in the DRC, which chose Option 1, with ZMapp + oSOC as the control arm.

Assessment

In the PREVAIL II trial, eligible subjects of any age were randomly assigned at a 1:1 ratio to receive either the current oSOC or the current oSOC plus three intravenous (IV) infusions of ZMapp (50 mg/kg, administered every third day). Subjects were stratified according to their baseline RT-PCR cycle-threshold (Ct) values (≤22 predicted a high viral load vs. >22) and by country of enrollment. The primary endpoint was the 28-day mortality rate. Due to curtailing of the outbreak in that region, a total of 72 subjects was enrolled at sites in Liberia, Sierra Leone, Guinea, and the United States, out of a desired accrual of 100 subjects per arm. Of the 72

subjects enrolled, only 71 were evaluated for the day-28 mortality endpoint and included in the analyses. Overall, 21 subjects died, for an overall case-fatality rate of 30%. Death occurred in 13 of 35 subjects (37%) who received the current oSOC alone and in 8 of 36 subjects (22%) who received the current oSOC plus ZMapp. The observed posterior probability that ZMapp plus the current oSOC was superior to the current oSOC alone was 91.2%, falling short of the prespecified threshold of 97.5%. Frequentist analyses yielded similar results (absolute difference in mortality with ZMapp, -15%; 95% CI, -36%, 7%). It was noted that the baseline viral load was strongly predictive of both mortality and duration of hospitalization in all age groups. Although the estimated effect of ZMapp appeared to be beneficial, the PREVAIL II trial result did not meet the prespecified statistical threshold for efficacy.

Table 15. PREVAIL II Summary of Results

Mortality of ZMapp+oSOC	Mortality of oSOC Alone	Bayesian Analysis: Posterior Probability Threshold (97.5%) for Superiority	Absolute Difference in Mortality (95% CI)
8/36 (22%)	13/35 (37%)	91.2%	-15% (-36%, 7%)

Source: (PREVAIL II Writing Group 2016)
Abbreviations: CI, confidence interval; oSOC, optimized standard of care

In summary, ZMapp demonstrated a numerically favorable trend over oSOC alone even though the PREVIAL II did not reach the level of statistical significance. Based on this information and given that the host country (DRC) preferred use of ZMapp + oSOC as the active control in the PALM trial, it was reasonable to use ZMapp as the control in the study instead of using system organ class alone.

Conclusion

Based on the preliminary experience with ZMapp in the PREVAIL II trial, the choice of ZMapp combined with oSOC is acceptable as an active control in the PALM trial. Although PREVAIL II did not meet the prespecified threshold, and was unable to establish a noninferiority margin for mortality, the use of an active control in the PALM trial was acceptable because of its superiority design. The results from the PALM trial are therefore interpretable and the trial design is adequate to demonstrate a superior benefit of REGN-EB3 versus ZMapp in terms of improvement in the 28-day mortality rate when combined with oSOC.

6.4.2. Lower Efficacy in Subjects With a Baseline CtNP of 22 or Lower

Issue

Lower efficacy was observed in subjects with high baseline EBOV viral loads (RT-PCR cycle threshold values using a nucleoprotein target [CtNP] ≤22) compared to subjects with CtNP >22, but it is unknown whether a higher dose would reduce mortality for those with high baseline EBOV viral loads (CtNP ≤22). This section summarizes the evaluation of nonclinical data in support of the human dose selection, including the limitations of the available nonclinical virology data.

Background

Given the challenges of conducting adequate and well-controlled trials for treatment of EBOV infection, the development program for REGN-EB3 was initially based on fulfilling the

necessary criteria for potential approval under the Animal Rule pathway. When the 2018 eastern DRC outbreak occurred, the nonclinical program was progressing but was incomplete. However, the NHP data were sufficient to support the proof-of-concept and use of a single 150 mg/kg IV dose of REGN-EB3 in the PALM trial and expanded access protocol (EAP). The NHP studies (rhesus macaques infected with EBOV) demonstrated improved survival of macaques treated with single doses of 100 mg/kg and 150 mg/kg compared to placebo (Section II.5.1). The 150 mg/kg dose was selected as the clinical dose for treatment of patients infected with EBOV in the PALM trial. At that time, the 150 mg/kg dose was considered a reasonable dose for use in the PALM trial based on the limited efficacy data in NHPs, PK in uninfected NHPs, and safety and PK in healthy subjects. PK in infected humans was not evaluated in the PALM trial, precluding evaluation of exposure-response relationships for efficacy or safety.

Assessment

PALM Trial: Increased Mortality in Subjects With High Baseline Viral Loads

In the PALM trial, REGN-EB3 was administered as a single IV infusion of 150 mg/kg, and subjects treated with this regimen had an overall mortality rate of 33.8% (52 of 154 subjects died) compared to 51.0% (78 of 153 subjects died) for the ZMapp control arm (p=0.002) (Table 16). As shown in Section II.6.3, the baseline CtNP value was a stratification factor and the mortality rates for subjects who had high baseline viral loads (CtNP ≤22) were 63.6% for REGN-EB3 and 87.5% for the ZMapp control arm (Table 16). The mortality rate was 11.4% for subjects treated with REGN-EB3 who had lower baseline viral loads (CtNP >22) compared to 25.0% for the ZMapp control arm (Table 16). The trial results demonstrate that subjects who exhibited lower viral loads at baseline generally experienced better outcomes. Despite the difference in mortality rates based on baseline CtNP values, REGN-EB3 was superior to ZMapp for both stratification factors.

Table 16. Twenty-Eight-Day Mortality Rate by Baseline Viral Load, PALM Trial, ITT Concurrent Population

Population/ Subpopulation	REGN-EB3 (N=154) Death/Total (%)	ZMapp (N=153) Death/Total (%)	Rate Difference % (95% CI)[a]	Boschloo's Two-Sided P-Value[b]
ITT Concurrent	52/154 (33.8%)	78/153 (51.0%)	-17.2 (-28.0, -4.1)	0.0023
CtNP at Baseline				
CtNP ≤22	42/66 (63.6%)	56/64 (87.5%)	-23.9 (-38.5, -7.3)	0.0015
CtNP >22	10/88 (11.4%)	22/88 (25.0%)	-13.6 (-25.3, -1.9)	0.0215

Source: Statistical reviewer, ADSL and SAS software were used.
[a] The exact confidence interval was based on inverting two one-sided tests in StatXact.
[b] P-value is based on Boschloo's test with a default gamma of 0 in StatXact.
Abbreviations: CI, confidence interval; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; N, number of subjects; PALM, PAmoja TuLinde Maisha

Multiple reviewers from different disciplines analyzed the data submitted by the Applicant to assess this review issue in detail. The Applicant has adequately demonstrated efficacy of 150 mg/kg of REGN-EB3 as a single IV dose for treatment of EBOV infection in humans, but the 52.2% absolute difference in mortality rates between subjects with high versus low baseline viral loads indicates that a higher dose may provide additional benefit for patients who present with higher baseline viral loads at the time of REGN-EB3 treatment.

Human Dose Selection was Based on Limited Nonclinical Data

The Applicant's demonstration of the antiviral activity of REGN-EB3 was predominantly based on an assessment of survival in exploratory rhesus macaque lethal challenge models that were incompletely characterized. They assessed various doses of REGN-EB3 and predicted that maximum antiviral activity would be observed in NHPs with a single dose of 100 mg/kg or higher (Section II.5.1). The 150 mg/kg dose for humans was initially selected to exceed exposures associated with 100 mg/kg in NHPs. The review team agreed with the Applicant's assessment and proposed dosing regimen for the PALM trial based on the totality of the data available at the time. However, the team also noted the lack of characterization of the NHP lethal challenge model used in these studies, as this assessment was pending the conduct of natural history studies. There are also several other limitations that preclude the NHP studies from being able to identify the optimal clinical dose (Section III.18). These limitations include an inability to power studies to determine differences between doses with activity, lack of confidence that all deaths in these studies are attributable to EBOV infection, and a lack of adequate evaluation of endpoints beyond mortality that may inform more-optimal dosing. Although the NHP models used in these studies are limited and do not provide a robust prediction of efficacy for humans across doses, some of the nonclinical virology data from these and later studies do support further dose exploration (Sections II.5.1 and III.18).

Dose Exploration Considerations Based on Nonclinical Virology Data

Higher Dose Exploration

The Applicant continued to consider the Animal Rule Pathway during clinical development in the event that the outbreak receded prior to completion of the PALM trial. In early NHP studies, the activity of REGN-EB3 was evaluated with doses up to 150 mg/kg (Sections II.5.1 and III.18). The Applicant evaluated 300 mg/kg in a later NHP study to confirm that the activity had reached a plateau with doses above 150 mg/kg. The analysis of this dose optimization study was incomplete; however, the viral kinetic data at 300 mg/kg showed a steeper decline in EBOV RNA than lower doses of REGN-EB3 (Section II.5.1). In the study, a comparison of the EBOV RNA decline from Day 5 (before initiation of treatment) to Day 8 showed a mean 1.2 $\log_{10}$ GE/mL decrease for the 150 mg/kg REGN-EB3 cohort (n=4 macaques) and a 2.1 $\log_{10}$ GE/mL decline for the 300 mg/kg REGN-EB3 (n=5 macaques) treatment cohort (Sections II.5.1 and III.18). Therefore, earlier NHP studies did not consider all of the relevant activity endpoints or evaluate a dose level high enough to define an optimal dose-response relationship, and therefore, a higher dose may improve outcomes for patients with higher viral loads (Sections II.5.1 and III.18).

Secreted GP Binding

The Applicant provided nonclinical data showing that odesivimab (REGN-3471) binds to full-length GP and secreted GP (sGP) with similar affinity, does not neutralize EBOV, and potentially exhibits ADCC activity. A potential explanation for the difference in efficacy between subjects with high and low baseline EBOV viral loads is that higher levels of sGP, which circulates at concentrations 100- to 1000-fold higher than the GP in EBOV particles in the serum of infected humans (Sanchez et al. 1996; Cook and Lee 2013; de La Vega et al. 2015), may reduce the effective concentration of odesivimab (REGN3471), which binds to virion-associated GP and sGP. Given that REGN-EB3 contains three mAbs that bind concomitantly to the EBOV GP, if the three mAbs provided overlapping efficacy, then reduction of odesivimab

(REGN-3471) due to sGP binding may not have an impact on the overall efficacy of REGB-EB3. However, if all three mAbs are required for efficacy, odesivimab (REGN-3471) binding to sGP may reduce the effective concentration of this mAb in the cocktail, reducing the overall effectiveness of REGN-EB3. The Applicant has not provided data showing the relative contribution of each mAb in REGN-EB3 in humans or a relevant animal model.

Data from two NHP studies indicate that sGP concentrations may impact the effectiveness of the REGN-EB3 cocktail. The main points of these studies are listed below and reviewed in the virology appendix (Section III.18).

1. **Study (b) (4) 2015-002** compared doses of 3×50 mg/kg, 2×50 mg/kg, and 1×150 mg/kg; n=4 or 5 per group in NHPs dosed starting at Day 5 postchallenge with IM 1,000 plaque-forming units (PFUs) of EBOV. In this study, baseline sGP concentrations were assayed.
    - NHPs with Day-5 EBOV titers >8 $\log_{10}$ GE/mL generally had considerably higher detectable sGP levels at Day 5.
    - NHPs that succumbed to EBOV infection had significantly higher Day-5 sGP levels than those that survived ($p<0.0004$). Mean Day 5 sGP concentrations for survivors were 18±5 µg/mL, compared to mean Day 5 sGP concentrations of 120±37 µg/mL in nonsurvivors.
2. **Study (b) (4) 2018-008** was a PK and PD study of 23 NHPs (4 or 5 per dose group) that received a single IV injection of 0, 30, 100, 150, or 300 mg/kg REGN-EB3 (mAbs at a 1:1:1 ratio) 5 days after challenge on Day 0 by IM injection with a target of 1,000 PFU EBOV.
    - The study assessed individual NHP mAb concentration-time profiles, Day 5 EBOV viral titers (RT-qPCR), Day 5 sGP levels, and survival data following IV infusion of 30, 100, 150, or 300 mg/kg REGN-EB3.
    - Day-5 EBOV viral titers trended with Day 5 sGP levels, with higher EBOV titers associated with higher Day 5 sGP concentrations and death.
    - Treated animals that succumbed to infection tended to have higher Day 5 EBOV titers and sGP levels relative to treated animals that survived infection.
    - Inconsistencies among Day 5 EBOV titers and sGP concentrations among NHPs made it difficult to assess the impact of a high Day 5 sGP concentration on treatment effect.

The data provided in the studies described above indicate that subjects with high baseline EBOV loads are more likely to have high baseline sGP concentrations, raising the concern that sGP may be acting as a decoy to absorb odesivimab (REGN-3471), thereby reducing its effectiveness and the overall effectiveness of the REGN-EB3 cocktail.

Incomplete Nonclinical Virology Development Program

Although REGN-EB3 was deployed in response to the 2018 outbreak in the DRC, the nonclinical virology development program for REGN-EB3 was incomplete, and had the following data uncertainties related to dose optimization:

1. The impact of sGP binding to odesivimab (REGN-3471) has not been adequately studied in cell-culture or animal-model antiviral activity assessments.

2. Mechanism of action studies with REGN-EB3 are incomplete; therefore, it is unknown to what extent the three mAbs contribute to antiviral activity .

The Virology reviewer recommended that the application is approvable based on the clinical trial results, and additional nonclinical information pertaining to resistance and mechanism of action may be addressed in post-marketing actions (III.22).

Summary of Data Uncertainities

Given the limitations of the EBOV NHP challenge model, the review team concluded that additional studies in NHPs are unlikely to resolve the dose-optimization question. The review team agreed that the most rigorous path forward would be to assess higher doses in future clinical trials of EBOV infection. There are two potential approaches to consider when assessing dose response:

- An increase in the concentration of all three mAbs in a 1:1:1 ratio cocktail may increase efficacy. The review team proposed that increasing the dose from 150 (50:50:50) mg/kg to 300 (100:100:100) mg/kg in patients who present with baseline EBOV viral loads CtNP ≤22 would enable assessment of higher doses. The Applicant has reported that RT-PCR is routinely used to confirm EBOV infection prior to dosing and that CtNP values are available for all subjects at the time of dose initiation.
- An increase in odesivimab (REGN-3471), which binds to sGP, may increase efficacy by increasing the amount of this mAb available to interact with sGP and GP in the EBOV particles of infected patients. In this scenario, the 1:1:1 ratio of mAbs would be altered to increase the concentration of odesivimab (REGN-3471), such as 1:4:1. The Applicant has declined to alter the ratio of mAbs in REGN-EB3.

Conclusion

The Applicant adequately demonstrated the efficacy of 150 mg/kg of REGN-EB3 as a single IV dose for treatment of EBOV infection in humans. A higher dose of REGN-EB3 may provide additional benefit to patients who are infected with EBOV and present with higher baseline viral loads. The review team reached consensus that a PMC would be communicated to the Applicant to further explore dose optimization for REGN-EB3. In the PMC we acknowledged the reliance on third parties to conduct the trial and requested the Applicant collaborate with US public health agencies, other public health agencies and local health authorities, as appropriate. The review team will work with the Applicant to develop a protocol and recognizes the Applicant's concerns about the number of subjects required for an adequately powered trial to assess two doses in adults and pediatrics patients with baseline CtNP values ≤22.

6.4.3. Demonstration of the Contribution of Each Component of the Combination

Issue

REGN-EB3 is a combination of three human mAbs directed against *Zaire ebolavirus* glycoprotein. Per 21 CFR 300.50 evidence is needed to show the contribution of each agent to the combination. In the absence of clinical studies evaluating the activity of each mAb versus the combination, other evidence is needed to satisfy 21 CFR 300.50.

Background

REGN-EB3 is a combination of three monoclonal antibodies (mAbs). All three mAbs are investigational drugs. FDA has published guidance for industry *Codevelopment of Two or More New Investigational Drugs for Use in Combination* (June 2013). This guidance applies only to drugs and biological products regulated by the Center for Drug Evaluation and Research.

When two or more drugs are combined in a single dosage form, each component must contribute to efficacy, otherwise patients may be exposed to risks of additional adverse reactions without the potential for benefit. 21 CFR 300.50 describes FDA's policy for the approval of fixed-combination prescription drugs for humans. The Federal Food, Drug and Cosmetics Act states in part, "Two or more drugs may be combined in a single dosage form when each component makes a contribution to the claimed effects and the dosage of each component (amount, frequency, duration) is such that the combination is safe and effective for a significant patient population requiring such concurrent therapy as defined in the labeling for the drug". The regulations are interpreted to require a factorial analysis of proposed combination ingredients to demonstrate the combination is more effective than each component of the combination alone. However, for REGN-EB3, a factorial design was not used and may not be appropriate because of the highly lethal nature of EBOV infection and components of the combination administered individually could lead to rapid development of resistance. Therefore, the evidence to show the contribution of each agent to the combination comes from the nonclinical program (Section II.5.1).

Nonclinical data can be used to support the contribution of each component to the efficacy of the combination if the individual contribution of each is not demonstrated by a clinical trial. Factorial designs may not be possible, such as combination products that have complimentary mechanisms of action and activity is not anticipated with each individual component.

Assessment

Because codevelopment generally will provide less information about the individual new investigational drugs, it may present greater risk compared to clinical development of an individual drug. Given these concerns, co-development is reserved for certain circumstances, and Ebola virus drug development would meet the criteria as outlined in the guidance for industry (June 2013).

Zaire ebolavirus disease is a serious, highly lethal disease and there is a strong biological rationale for use of the combination to reduce resistance, which can lead to treatment failure. Nonclinical studies show that all three REGN-EB3 mAbs bind sequentially and simultaneously to EBOV Makona GP and have antiviral activity. Maftivimab (REGN3479) neutralizes several EBOV strains (Makona, Mayinga, and Kikwit). Atoltivimab (REGN3470) and odesivimab (REGN3471) induced FcγR3A-mediated antibody-dependent cellular phagocytosis. In addition, atoltivimab (REGN3470) exhibited neutralization activity in pseudovirus assays. Atoltivimab (REGN3470) and odesivimab (REGN3471) bind to proximal epitopes on the EBOV glycoprotein surface with some overlap of their footprints, but do not overlap the footprint of maftivimab (REGN3479).

Viruses within an infected individual exist as a heterogeneous population with one in 1,000 to one in 10,000 genomes containing a resistant mutation expected for any one of the mAbs. The above nonclinical data indicate that each mAb has unique resistance mutations such that a combination of unique resistance mutations for all three mAb would be less likely to occur

together within a single virus genome in the virus population. Viruses in the population having one or two of the unique resistance mutations within their genome would be eliminated by the other mAb(s).

Using a combination of three mAbs in the cocktail is expected to reduce the likelihood that the efficacy would be impacted by resistance. Additional postmarketing resistance assessments will further characterize resistance and cross-resistance.

Conclusion

The Applicant has fulfilled the requirements of 21 CFR 300.50 and provided nonclinical data to indicate that each mAb contributes to the efficacy of the combination.

6.4.4. Adequacy of Clinical Experience With Pediatric Subjects and Inclusion of Labeling for Low-Birth-Weight Neonates Born to EBOV-Infected Mothers

Issue

Although the PALM trial and EAP 1846 included subjects of all ages, including neonates born to mothers infected with EBOV, there was limited experience with subjects less than 18 years of age, particularly with neonates less than 1 month of age. Additionally, while REGN-EB3 dosing recommendations are based on weight, there remains a lack of PK data from infected subjects to inform optimal dosing for all weight ranges.

Background

On July 14, 2016, REGN-EB3 was granted Orphan Drug Designation (#16-5363) for the treatment of patients with EBOV infection. With this designation, Pediatric Research Equity Act (PREA) requirements were exempted. Nevertheless, given the anticipated benefit (supported by initial NHP studies) and the high mortality rate associated with untreated EBOV infection, the weight-based dose rationale was considered acceptable for enrollment of pediatric subjects in the PALM trial and EAP 1846. Neonates ≤7 days of age were eligible if the mother had documented infection, including if the mother had cleared her infection but the investigator thought the neonate was likely to be infected.

Assessment

Table 17 shows enrollment by age group in the PALM trial and EAP 1846. Overall, 77 subjects (20%) of the combined population were in pediatric age groups.

Table 17. Subjects Treated With REGN-EB3 by Age Group, PALM Trial and EAP 1846

Age Group	PALM RCT (N=154)	EAP 1846 (N=229)	Total (N=383)
<1 month	1 (0.6%)	2 (0.9%)	3 (0.007%)
1 month to <1 year	4 (2.6%)	7 (3.1%)	11 (3%)
1 year to <5 years	17 (11%)	11 (4.8%)	28 (7%)
5 to <12 years	9 (5.8%)	7 (3.1%)	16 (4%)
12 to <18 years	8 (5.2%)	11 (4.8%)	19 (5%)
<18 years	39 (25%)	38 (17%)	77 (20%)
≥18 years	115 (75%)	191 (83%)	306 (80%)

Source: Reviewer analysis
Abbreviations: EAP, expanded access protocol; N, number of subjects; PALM, PAmoja TuLinde Maisha; RCT, randomized controlled trial;

The primary review division, Division of Antivirals (DAV), placed an interoffice neonatal-perinatal medicine consultation request to the Office of Pediatric Therapeutics, OCPP/OC. In her responding memorandum, Gerri Baer, MD noted that in the PALM trial, 39 of the 154 subjects (25%) who received REGN-EB3 were <18 years of age, with the largest proportion (n=18) 1 year to <6 years of age. One subject was <1 month of age, and four subjects were 1 month to <1 year of age. The enrolled neonate was 14 days of age, with multiorgan failure prior to enrollment. Unfortunately, he died the day after treatment with REGN-EB3. The mortality rate by age group in the PALM trial is shown in Table 18. Overall, the mortality rate was consistent in pediatric (33%) and adult (34%) subjects.

Table 18. PALM Trial Mortality Rate

Age Group	REGN-EB3 n/N (%)	ZMapp n/N (%)
<1 month	1/1 (100%)	0/2
1 month to <1 year	2/4 (50.0%)	0/1
1 year to <5 years	7/17 (41.2%)	6/12 (50.0%)
5 to <12 years	1/9 (11.1%)	1/5 (20.0%)
12 to <18 years	2/8 (25.0%)	4/8 (50.0%)
<18 years	13/39 (33.3%)	11/28 (39.2%)
≥18 years	39/115 (33.9%)	67/125 (53.6%)

Source: Reviewer analysis
Abbreviations: N, number of subjects; n, number of subjects in subgroup; PALM, PAmoja TuLinde Maisha

In EAP 1864, the mortality rate was 40% (15/38) in pediatrics compared to 30% (57/190) in adults. The pediatric population included two neonates and seven infants 1 month to <1 year of age. Of the nine subjects <1 year of age, four (including one of the two neonates) died, for a mortality rate of 44.4%. Of the entire population enrolled in EAP 1846, the mortality rate was 31.4%, similar to the results with REGN-EB3 in the PALM trial.

Conclusion

Overall, REGN-EB3 demonstrated a significant mortality benefit over an active control in the pediatric population. (b) (4) the primary review team asked the Office of Pediatric Therapeutics to comment on the inclusion of a minimum weight in the product indication, specifically inclusion of extremely low-birth-weight neonates given available study data. DAV and the Office of Pediatric Therapeutics agreed that the INMAZEB label should provide dosing and administration information to address all populations for which the potential benefits of the product outweigh the potential risks, including preterm neonates with a minimum weight of 0.5 kg.

Specific safety concerns related to dosing and administration are further discussed in Section II.7.7.3 as review issues related to the assessment of risk.

6.4.5. Lack of Clinical Experience With REGN-EB3 for Treatment of EBOV Infection Acquired by Routes Other Than Natural Transmission

Issue

The proposed indication for Inmazeb (REGN-EB3) is "for the treatment of infection caused by *Zaire ebolavirus* in adult and pediatric patients, including neonates born to a mother who is RT-

PCR positive for *Zaire ebolavirus* infection". There is the potential for this drug to be used in the United States to treat other transmission routes, for example an occupational exposure or by intentional release. However, the PALM trial and MEURI EAP treated subjects infected with EBOV who were presumably infected via the natural EBOV transmission route (i.e., contact with infected blood or body fluids).

Background

Clinical data for REGN-EB3 efficacy are limited to presumed natural-infection of EBOV, although additional data from occupational exposures acquired during the MEURI EAP may be available in the future.

Studies in lethal NHP challenge models of EBOV have indicated that the 150 mg/kg dose of REGN-EB3 administered by IV 5 days after challenge with 1,000 PFU of EBOV administered by IM reduces mortality (Section II.5.1); however, the 1,000 PFU challenge dose is likely a much lower dose than would be delivered in a needlestick exposure (Geisbert et al. 2002; Günther et al. 2011).

Nonclinical studies would be needed to support efficacy for an intentional release and would depend on the type of release (i.e., route of exposure, exposure dose, etc.).

Assessment

The review team discussed this issue at length, and considered potential label changes (in red) to address the final wording of the indication, including:

1. **Changing the indication:** INMAZEB is a combination of (b) (4) monoclonal antibodies (atoltivimab, maftivimab, and odesivimab), (b) (4) indicated in adult and pediatric patients for the treatment of naturally acquired infection caused by *Zaire ebolavirus*.
2. **Adding a limitation of use:** The efficacy of INMAZEB has not been established for *Zaire ebolavirus* infection caused by unnatural routes of exposure (i.e., needlestick, or intentional release)

(b) (4)

Conclusion

Clinical Review Team Perspective

The consensus of the Clinical review team for REGN-EB3 was to not address this issue with a Limitation of Use statement or with a modification to the indication in the proposed label. The Clinical review team agreed that although the NHP studies were inadequate to demonstrate evidence of efficacy in the setting of needlestick or intentional release, restricting to "naturally acquired" infection could result in delay or deferral of use despite evidence that there may be some benefit in the context of needlestick exposure or other healthcare-associated exposures.

Clinical Virology Perspective
While Clinical Virology agrees that REGN-EB3 (or TRADENAME) should be approved based on the clinical results the indication should state "*naturally acquired*" infection given that a needlestick exposure, which may occur at markedly higher concentrations of EBOV, was not studied and the EBOV disease course is likely to be significantly different in the event of an intentional release of EBOV.

Signatory Perspective
After reviewing the totality of the data, it is reasonable to conclude that REGN-EB3 may offer benefit in various exposure settings, and the Clinical Review Team perspective regarding labeling is accepted. Needlestick exposures could have variable concentrations of EBOV, as could nefarious events. Depending on the level of exposure, REGN-EB3 may mitigate disease and thereby offer benefit in combination with standard of care. Therefore, labeling that would delay or limit this off label use does not seem appropriate.

7. Risk and Risk Management

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

The nonclinical safety profile of REGN-EB3 supporting this BLA was evaluated in (1) a 3-week repeated-dose toxicity study in rats, and (2) tissue cross-reactivity studies in human and rat adult and human fetal tissues. Additional toxicology studies, including genotoxicity, carcinogenicity, and developmental and reproductive toxicology, were not conducted since they are not considered applicable and/or warranted. PK/toxicokinetics were studied as part of the toxicity study and in separate animal PK studies in monkeys (both uninfected and infected with EBOV).

No target organs of toxicity or off-target tissue binding of the mAb(s) were identified, and systemic pharmacologic effects did not suggest a potential risk to humans.

See Section III.13 for additional details on the nonclinical drug development program for REGN-EB3.

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

As with all therapeutic proteins, there is potential for immunogenicity. Treatment of patients with therapeutic protein products, such as monoclonal antibodies, may result in immune responses of varying clinical relevance based on product- and patient-specific factors.

Because REGN-EB3 contains proteins that could induce antibody formation, antidrug antibodies (ADAs) were measured in Study 1528 (NCT02777151). Although no ADAs (anti-REGN3470, anti-REGN3471, or anti-REGN3479 antibodies) were detected in Study 1528, this does not rule out potential clinical consequences of immunogenicity, because the presence of ADA alone is not necessarily predictive of anaphylaxis or other hypersensitivity reactions. Additionally, the detection of ADAs is highly dependent on the sensitivity and specificity of the assay and may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease.

Therefore, when considering collection of safety data in the PALM trial, *infusion-related reactions* were included as an important range of adverse drug reactions (ADRs) that were prespecified in the schedule of safety assessments. Although the term *infusion reaction* implies a certain temporal relationship, they are otherwise not well-defined. Furthermore, immune responses secondary to immune complex formation typically have a subacute presentation. These reactions may include fever, rash, arthralgia, myalgia, hematuria, proteinuria, serositis, central nervous system complications, and hemolytic anemia (Hunley et al. 2004; Goto et al. 2009). In the setting of acute EBOV infection, however, these potential reactions are highly confounded by the signs and symptoms of the underlying disease. These potential reactions are explored in detail in Section II.7.6.5.

7.3. Potential Safety Concerns Identified Through Postmarketing Experience

REGN-EB3 has not been approved in any country; therefore, there has been no postmarketing experience with REGN-EB3.

7.4. FDA Approach to the Safety Review

Adequacy of Applicant's Clinical Safety Assessments

Due to the challenges of data collection, the PALM trial was designed with a reduced data collection plan. The sponsor of the trial, NIAID, noted in the protocol: "Every attempt will be made to document the nature [name/type] and the severity [grade per DAIDS toxicity table version 2.1, July 2017] of conditions present at baseline, particularly as pertains to the status of Ebola infection and vital organ function, so that meaningful data can be collected on the safety and efficacy impact of study interventions. It is acknowledged at the outset that this effort will likely be incomplete, and there may be unavoidable inconsistencies over time and from place to place, due to harsh conditions at treatment/study sites."

On Sunday February 24, 2019 there was an attack on the study team, resulting in a fire, at the Katwa Ebola Treatment Centre. Various infrastructure and study supplies were destroyed. Another attack and fire occurred on Wednesday February 27, 2019 at the Butembo Ebola Treatment Centre resulting in building destruction and major material damage. Some case report form (CRF) binders (paper copies) were lost during the fire; however, scanned copies of these CRFs were retained. Médecins Sans Frontiéres (MSF), which was providing staffing for these facilities, withdrew their personnel after these events.

According to the 19-I-0003 PALM RCT protocol, study data collected at the bedside at study sites were to be later recorded as paper or electronic CRFs with subsequent transmission to the Data Coordinating Center. Data Coordinating Center personnel entered the data into an electronic database. Corrections to electronic data systems were to be tracked electronically (password protected and through an audit trail) with time, date, individual making the correction, and the nature of the change. Reports containing French terms were submitted to a certified vendor for authorized translation into English. In addition, any pertinent documentation (i.e., protocol and pharmacy manual, informed consent form [ICFs]) sent to the DRC by the NIAID was translated into French. Vital signs, signs and symptoms, optional procedures data, and supportive care were not queried. The chemistry laboratory data for the screening visit were

reviewed and compared with source documents; however, no further information on the monitoring of chemistry laboratory data was provided.

After the final database review and inspections, NIAID allowed the sponsors of REGN-EB3 (Regeneron) and mAb114 (Ridgeback) to submit queries. Although DAV requested that NIAID assure that data met Clinical Data Interchange Standards Consortium (CDISC) standards before sharing with Regeneron and Ridgeback, NIAID declined because NIAID was not directly responsible for submitting either BLA. After the database was locked, only the data from the REGN-EB3 and ZMapp arms were transferred to the Applicant for this BLA. Regeneron then converted their respective datasets independently.

The Applicant proposed presentation of ADRs from Study 1528 (a phase 1 healthy volunteer study) in lieu of the safety data from the PALM trial; however, this study will not be considered essential to the assessment of safety for the proposed indication, because only six volunteers received REGN-EB3 at the proposed dose. Additionally, the interaction of REGN-EB3 with the underlying EBOV infection was considered critical for the assessment of safety for the proposed treatment indication.

The initial BLA submission included data from the completed PALM RCT main phase and the ongoing EAP 1846. No data were included for the PALM RCT extension phase (initiated 10 August 2019), which evaluated subjects randomized to receive either REGN-EB3 or mAb114. Data from the PALM RCT covered enrollment through August 9, 2019 (database locked January 17, 2020), and data from the EAP covered outcomes as of September 20, 2019. From February 17, 2020 to April 3, 2020, no new cases of EBOV infection were reported in the DRC. However, on April 10, 2020, a new confirmed case was reported. The Applicant submitted a 60-day Safety Update Report on April 24, 2020, which covered corresponding interval data for each study until April 3, 2020; however, mortality data from the extension phase of the PALM RCT will not be shared with any third party until completion of the study.

Approach to Assessment of Clinical Trial Data

This review of clinical safety considers all of the challenges of data collection inherent with EBOV outbreaks and the sociopolitical challenges caused by the location of the outbreak.

Because mortality was the primary efficacy endpoint of the PALM RCT, the assessments of benefit and risk overlap. With the demonstration of a statistically significant treatment effect on mortality, a degree of uncertainty with the assessment of safety can be accepted. Prespecified testing was not proposed for any safety outcomes. Comparisons between treatment arms in the PALM trial, however, are based on descriptive analyses. Pooling of the data of the PALM trial with the EAP was not feasible due to significant differences in data collection (the EAP had incomplete and unstructured data reporting, lack of causality assessments, and challenges in obtaining follow up information).

Clinical trial data were independently analyzed using JMP and JReview software. Additional analyses were provided by the Clinical Data Scientist support team. All safety assessments and conclusions are those of the clinical review team unless otherwise specified.

7.5. Adequacy of the Clinical Safety Database

Overall, the safety database is adequate to assess the safety of REGN-EB3 for the proposed indication, dosage regimen, and patient populations. Table 19 summarizes the clinical safety data available for evaluation.

Table 19. Overview of Clinical Safety Data

Study	Description	Number of Subjects
19-I-0003 (PALM RCT) (data cutoff 9 Aug 2019*)	OL, RCT	Safety population: REGN-EB3=154, ZMapp=168 (postdischarge follow-up of 58 days).
19-I-0003 (PALM Extension Phase) (data cutoff 03 Apr 2020)	OL, RCT	An estimated 180 have received REGN-EB3 (Assuming 1:1 randomization of 359 subjects. Safety reported only as SUSAR and pregnancies)
Study 1846 (EAP 1846) (data cutoff 20 Sept 2019)	MEURI EAP	N=229
Study 1528	P1 FIH, HV	REGN-EB3=18 (N=6 at proposed dose), N=6 placebo
Total REGN-EB3 Safety Database		**N=581**

Source: Reviewer's analysis
*On August 10, 2019, four subjects switched from ZMapp or remdesivir to REGN-EB3 due to stopping of the PALM RCT.
Abbreviations: EAP, expanded access protocol; FIH, first in human; HV, healthy volunteer; MEURI, Monitored Emergency Use of Unregistered and Investigational Interventions; N, number of subjects; OL, open label; PALM, PAmoja TuLinde Maisha; RCT, randomized controlled trial; SUSAR, suspected unexpected serious adverse reaction

The PALM RCT and EAP 1846 differed in the methods used for the collection of safety information. The PALM RCT was randomized and systematically collected safety data from all treatment groups using CRFs. PALM also provided a SOP to define SAEs as events not thought to be related to the underlying EBOV infection. Conversely, EAP 1846 was not randomized, lacked a comparator treatment group, did not have criteria for SAEs specified in the protocol, and lacked verifiable investigators documented at the Ebola treatment units (ETUs). The protocol did not specify safety data collection and the designated CRFs were not completed and returned to the Applicant. Additionally, the limited ability to communicate with the sites further diminished the capacity to collect information. Therefore, the safety data from EAP 1846 will not be integrated with the PALM RCT for this review. Instead, the PALM RCT is provided as the primary assessment of safety, and EAP 1846 serves as supportive data. When available, supplemental analyses from EAP 1846 are provided in the following respective sections where available.

In the PALM trial, there was adequate assessment of exposure with REGN-EB3, as REGN-EB3 was intended to be administered as a single infusion (whereas subjects in the ZMapp and remdesivir arms required multiple infusions). Exposure and treatment duration are summarized in Table 20.

Table 20. Duration of Exposure, Safety Population, PALM RCT

	REGN-EB3 150 mg/kg N=168	ZMapp N=154
Total number of doses administered	154	361
Infusions not administered completely	2 (1.3%)	6 (1.7%)
Any duration (including partial infusion)	168 (100%)	154 (100%)
≥2	0	105 (62.5%)
≥3	0	88 (52.4%)

Source: Applicant's 19-I-0003 post-text table
Abbreviations: N, number of subjects; PALM, PAmoja TuLinde Maisha; RCT, randomized controlled trial;

Six subjects in the ZMapp group did not complete at least one of the three infusion doses because of issues that occurred during infusion. One subject died 40 minutes after the end of her first infusion, one subject died 15 minutes after the beginning of his first infusion, and one patient died 23 minutes after the beginning of her first infusion.

Table 21 summarizes the duration of observation following receipt of study drug in the PALM RCT. The longer duration of observation in the REGN-EB3 arm was largely driven by the higher rate of survival.

Table 21. Summary of Study Duration, Safety Population, PALM RCT

Parameter	REGN-EB3 150 mg/kg N=168	ZMapp N=154
Duration of observation period[a] (units)		
Mean (SD)	42.1 (25.6)	31.6 (27.7)
Median (min, max)	58.0 (1, 68)	48.5 (1, 66)
Duration of observation period, n (%)		
≥1 day	154 (100%)	168 (100%)
≥14 days	107 (69.5%)	87 (51.8%)
≥29 days	107 (69.5%)	85 (50.6%)
≥59 days	52 (33.8%)	40 (23.8%)

Source: Applicant's 19-I-0003 post-text Table 14.1.6.2
[a] Duration of the observation period was defined as (death date or last known alive date − date of randomization) +1.
Abbreviations: N, number of subjects; n, number of subjects in subgroup; PALM, PAmoja TuLinde Maisha; RCT, randomized controlled trial; SD, standard deviation

In EAP 1846, 229 subjects were enrolled and treated with REGN-EB3 under Monitored Emergency Use of Unregistered and Investigational Intervention (MEURI) at seven ETU locations (Beni, Butembo, Katwa, Mangina and Komanda, Chowe CS, and Goma) in one country (DRC). Subject disposition is summarized in Table 22.

Table 22. Summary of Disposition, Safety Population, EAP 1846

Disposition, n (%)	REGN-EB3 N=229
All treated subjects	229 (100%)
Subjects who were discharged	156 (68.1%)
Subjects who died	72 (31.4%)
Subjects who were ongoing as of data cutoff date	1 (0.4%)

Source: Applicant's Clinical Study Report for EAP 1846, Table 2. Data cutoff date September 20, 2019
Abbreviations: EAP, expanded access protocol; N, number of subjects; n, number of subjects in subgroup

7.6. Safety Findings and Safety Concerns Based on Review of the Clinical Safety Database

EBOV infection is associated with significant clinical and laboratory presentations that confound assessment of the safety of drugs administered to treat active disease. In subjects treated with REGB-EB3, the observed safety profile was largely consistent with the expected clinical presentation of EBOV infection.

7.6.1. Overall Adverse Event Summary

For the PALM Trial, only SAEs were recorded and summarized. The observation period was divided into three segments: pretreatment, treatment, and posttreatment. The pretreatment period was defined as the time elapsed from when the subjects gave informed consent and the start of

the investigational product. The treatment period was defined as the time from the first dose of investigational product to 58 days after the last dose. The posttreatment period was defined as starting 58+1 days after the last dose of investigational product (after the on-treatment period). SAEs from each observation period were provided as data when available. Day 1 was the first day of the investigational product and day –1 was the day before; there was no Day 0.

Pretreatment SAEs were defined as SAEs that developed or worsened during the pretreatment period.

Treatment-emergent SAEs were defined as SAEs that developed or worsened during the on-treatment period.

Posttreatment SAEs were defined as SAEs that developed or worsened more than 58 days after the last dose of investigational product and were not considered drug related by the investigator.

The PALM RCT included the requirement that an event for a subject must be assessed as not related to their underlying EBOV infection or related to the study drug to be considered as an SAE. Further, when an SAE was identified, two assessments of relatedness to study medication were performed, one by the site investigator and the other by the pharmacovigilance working group.

The Safety Analysis population included all subjects who received either ZMapp or REGN-EB3 and were analyzed as treated (that is, if a subject received the wrong treatment, they were analyzed as to their actual treatment assignment). The Safety Analysis population was used for all safety analyses. Subjects who first received ZMapp or remdesivir and who were subsequently switched to REGN-EB3 after August 9, 2019 based on DSMB recommendations are included in tables based on the drug received according to the initial randomization.

Table 23. Overview of Adverse Events, Safety Population, PALM Trial

Subjects experiencing at least one event	REGN-EB3 N=154	ZMapp N=168
Any nonserious adverse event[1]	120 (77.9%)	151 (89.9%)
Any SAE	6 (3.6%)	3 (1.9%)
Any treatment-emergent SAE[2]	4 (2.4%)	2 (1.3%)
SAEs with fatal outcome	1 (0.6%)	1 (0.6%)
SAE leading to discontinuation of study drug	0	1 (0.6%)
SAE related to study drug	2 (1.2%)	0

Source: Reviewer's analysis of the adae.xpt and adfa2.xpt datasets using JReview
[1] Includes only events reported as adverse drug reactions that occurred during or on the day of infusion
[2] Treatment-emergent SAE is defined as any event occurring up to 58 days after the last dose of investigational product
An assessment of relationship with the study drug was not performed for all nonserious adverse events (AEs). Rather, the CRF included prespecified symptoms l kely to be related to either infusion-related events or symptoms common in EBOV disease. Therefore, the Applicant submitted this additional safety data in the standard (SDTM) "Findings About" (fa.xpt) and analysis (ADaM) (adfa.xpt) datasets, and the "Adverse Events" datasets did not include any nonserious AEs. The "Findings About" datasets included reported "adverse drug reactions," which were verbatim terms recorded by the investigator. These terms descr bed the signs and symptoms that occurred during and immediately after infusion of the study drug on the day of infusion. Note that for the ZMapp arm, this occurred on two subsequent days for the second and third doses, respectively, whereas REGN-EB3 was assessed with only the single infusion on baseline Day 1.
Abbreviations: N, number of subjects in group; n, number of subjects with at least one event; PALM, PAmoja TuLinde Maisha; SAE, serious adverse event

An assessment of relationship with the study drug was not performed for all nonserious adverse events (AEs). Rather, the CRF included prespecified symptoms likely to be related to either infusion-related events or symptoms common in EBOV disease. Therefore, the Applicant submitted this additional safety data in the standard (SDTM) "Findings About" (fa.xpt) and analysis (ADaM) (adfa.xpt) datasets, and the "Adverse Events" datasets did not include any

nonserious AEs. The "Findings About" datasets included reported "adverse drug reactions," which were verbatim terms recorded by the investigator. These terms described the signs and symptoms that occurred during and immediately after infusion of the study drug on the day of infusion. Note that for the ZMapp arm, this occurred on two subsequent days for the second and third doses, respectively, whereas REGN-EB3 was assessed with only the single infusion on baseline Day 1.

The terms listed in the fa.xpt and adfa.xpt datasets were not associated with some metadata typically associated with adverse events (i.e., no assessment of causality, severity, or grade). The terms were also not standardized to Medical Dictionary for Regulatory Activities (MedDRA) terminology by the primary sponsor (NIAID). A number of terms were combined under a miscellaneous term "Other adverse reactions (ARs)."

An Request for Information was sent to the Applicant on March 13, 2020, (b) (4) The Applicant was requested to revise section 6 of the proposed label with the terms from the "Findings About" datasets grouped under the category "Other."

7.6.2. Deaths

Deaths are discussed in Section II.6.3 as a primary efficacy endpoint. One death in the REGN-EB3 arm, subject (b) (6) was due to a treatment-emergent SAE. Complete CRFs and narratives were only available for REGN-EB3 subjects for this review.

Subject (b) (6) was a 55-year-old female in Butembo with confirmed EBOV disease. She had 9 days of symptoms prior to treatment and presented at screening with fever, headache, vomiting, diarrhea, abdominal pain, conjunctival injection, asthenia, and anorexia. Prior to treatment with REGN-EB3, her ALT was 69 U/L (high), creatinine was 0.9 mg/dL (normal), and her CtNP was 28. Her signs and symptoms during or after infusion included chills, elevation in fever, hypertension, and vomiting. She completed the study infusion, was discharged from the ETU on Day 8, and completed the Day 28 follow-up visit at which she showed normalized sodium, potassium, and ALT levels and a negative RT-PCR for EBOV. She was given antimalarials at the Day 28 visit, but no hematology laboratories were available. She died on Day 40. The family reported that the death was due to anemia and lower extremity edema. This event was assessed by the investigator to be unrelated to REGN-EB3. This reviewer's assessment is inconclusive without confirmation of a unifying or underlying diagnosis for "anemia" and "lower extremity edema."

7.6.3. Serious Adverse Events

SAEs are summarized in Table 24.

Table 24. Serious Adverse Events, Safety Population, PALM Trial

Actual Arm	Unique Subj ID	AE Start Day	SAE Start Day	Preferred Term	Outcome
REGN-EB3	(b) (6)	129	129	Ebola disease	Recovered/resolved
REGN-EB3		-5	25	Pulmonary tuberculosis	Recovered/resolved
REGN-EB3		36	40	Anemia	Fatal
ZMapp		1	2	Diarrhea	Fatal
		1	2	Vomiting	Fatal
ZMapp		157	157	Hydrocephalus	Not recovered/not resolved
		157	157	Umbilical cord short	Not recovered/not resolved
ZMapp		1	1	Anaphylactic shock	Fatal
ZMapp		96	96	Fetal death	Not recovered/not resolved
ZMapp		14	19	Edema peripheral	Recovered/resolved with sequelae
ZMapp		2	9	Urethral injury	Recovered/resolved

Source: Reviewer's analysis of the ae.xpt dataset using JReview
Coded as MedDRA preferred terms
Abbreviations: AE, adverse event; PALM, PAmoja TuLinde Maisha; SAE, serious adverse event

In the REGN-EB3 arm, three subjects had SAEs; two were nonfatal (Subjects (b) (6) and (b) (6)). This reviewer agrees with the investigator's and Applicant's assessments that these two nonfatal SAEs were not related to REGN-EB3. Subject narratives are provided below.

Subject (b) (6) was a 26-year-old male in Beni with confirmed EBOV disease and a suspected baseline diagnosis of tuberculosis. He had 5 days of symptoms prior to treatment and presented with fever, vomiting, diarrhea, conjunctival injection, dysphagia, and vertigo. Prior to treatment with REGN-EB3, he had an ALT of 1433 U/L (high), creatinine of 1.1 mg/dL (normal), and CtNP of 20.0. Signs and symptoms reported during or after infusion included elevation in fever, rigors/tremors, diarrhea, vomiting, and headache. On Day 25, miliary pulmonary tuberculosis was reported. On Day 50, the subject returned to the ETU with episodes of chronic fever and dyspnea but had a negative RT-PCR for EBOV. This event was reported as a treatment-emergent SAE (onset Day 25). The subject was alive at the Day 58 follow-up and was reported to have recovered from tuberculosis.

Subject (b) (6) was a 29-year-old female in Beni with confirmed EBOV disease. She had 5 days of symptoms prior to treatment and presented with diarrhea. She was pregnant on admission (gravidity of 3 and parity of 2). Prior to treatment with REGN-EB3, she had an ALT of 36 U/L (normal), creatinine of 0.6 mg/dL (normal), and CtNP of 29.6. The signs and symptoms reported during or after infusion included rigors/tremors, vomiting, and epigastralgia. She completed the study infusion, all of the planned study assessments, and was alive at the Day 58 follow up. She gave birth 128 days after treatment with REGN-EB3 at 42 weeks of gestation. A sample from her placenta was positive for EBOV by RT-PCR (CtNP 25.7 and cycle-threshold glycoprotein gene targets [CtGP] 27.9), as were the samples from the umbilical cord, amniotic fluid, and salivary swab. The event of Ebola disease (in newborn) was reported as a nontreatment-emergent SAE (onset Day 129). No other congenital anomaly or malformation was noted at birth. The newborn was randomized to receive another investigational product (mAb114) on the day of birth. Ebola RT-PCR on an infant blood sample was negative 1 day after treatment with the investigational product. Additional details of the outcomes of the pregnancy and the newborn are provided in Section II.8.4 (Table 31).

Although this SAE was not considered related to REGN-EB3, this case illustrates the potential limited efficacy and risk of long-term sequelae even after successful treatment of the initial clinical presentation of EBOV infection. Monoclonal antibodies cross the placenta to the fetus; however, the virus may be able to persist in privileged sites, including products of conception, enabling transmission to the fetus several weeks to months after recovery (Baggi et al. 2014). Long-term follow up beyond 58 days was not available for many other subjects, so there is limited collection of potential other long-term sequalae due to EBOV persistence in privileged sites; e.g., uveitis or orchitis (Vetter et al. 2016).

In the 60-day safety update, one suspected unexpected serious adverse reaction of "seizure" (subject (b) (6) in the PALM RCT) was reported. This was a delayed report of a 28-year-old male who experienced the event on (b) (6). He had a 4-day history of EBOV-related symptoms and presented with hypovolemic shock. Shortly following initiation of REGN-EB3, the subject experienced a generalized tonic-clonic seizure. The infusion was stopped, and the subject received phenobarbital and a glucose solution. Approximately 20 minutes later, the infusion was restarted, and the subject experienced another seizure episode, which resulted in permanent discontinuation of the study drug. Given the timing and observed positive rechallenge, the PALM pharmacovigilance working group assessed the seizure to be possibly related to REGN-EB3, though it was noted that this seizure phenomenon is also seen in patients with EBOV infection. The subject subsequently expired the same day with multiorgan failure and hypovolemic shock, which were assessed as consistent with and most likely caused by EBOV infection.

Although this case was concerning for the close temporal relationship of the infusion and recurrence with a subsequent rechallenge, this reviewer agrees that definite causality cannot be assigned to REGN-EB3. Seizure has been previously associated with EBOV infection, as noted, and may have been refractory to pharmacologic treatment.

7.6.4. Dropouts and/or Discontinuations Due to Adverse Events

Two subjects (1.3%) in the REGN-EB3 arm of the PALM RCT did not receive their complete infusion because of issues that occurred during infusion. The first subject was a 48-year-old female subject who experienced an elevation in fever during infusion, had the infusion rate slowed, was treated with oxygen and antipyretics, and did not complete the infusion. The second subject was an 8-month-old female subject (no other information recorded). Both subjects died on the day of infusion.

In EAP 1846, there were two cases (0.9%) in which the investigator noted possible "anaphylactic shock", which resulted in premature discontinuation of REGN-EB3 infusion. The first case was subject (b) (6) a 71-year-old male who experienced hypotension, chills, and diarrhea during infusion. The investigator described this as "anaphylactic shock." REGN-EB3 was interrupted, and the subject was treated with epinephrine. The infusion of REGN-EB3 was restarted at a slower rate, and the subject was able to complete the dose with minimal complaints (nausea).

The second case was subject (b) (6) a 36-year-old male who experienced chest pain, dyspnea, chills, and hypotension approximately 15 to 30 minutes after the start of the infusion. The infusion was stopped, and the subject received epinephrine due to suspected anaphylactic shock; he was reported to show improvement afterwards. The infusion was not

restarted because the subject expired on the next day due to EBOV infection, as assessed and documented by the investigator.

7.6.5. Treatment-Emergent Adverse Events

In the initial proposed label, the Applicant included only data from the phase 1 first-in-human, healthy volunteer study (Study 1528). This was a randomized, double-blind, placebo-controlled, dose escalation (3 mg/kg, 15 mg/kg, 60 mg/kg, and 150 mg/kg, or placebo). Study 1528 was conducted to investigate the safety, tolerability, immunogenicity, and PK of REGN-EB3 in 24 healthy adults. Eighteen volunteers received REGN-EB3 and six received placebo, but only six subjects (Cohort 4) received the full proposed dose of 150 mg/kg. In this small subset, four of six subjects (66.7%) were reported to have had at least one treatment-emergent adverse event (TEAE). The most common TEAE was headache, which was reported in three subjects (50.0%) and was considered related to the study drug in two subjects (33.3%). In the placebo group, three subjects (50.0%) experienced at least one TEAE. None of the TEAEs in the placebo group were considered related to the study drug.

Although Study 1528 was not confounded by underlying EBOV infection, the sample size was too small to make an adequate assessment of the safety of REGN-EB3. The study population (only healthy men and women, 21 to 60 years of age) was also inadequate to accurately describe the anticipated clinical safety outcomes in EBOV-infected patients.

A Request for Information was sent to the Applicant on March 13, 2020 (b) (4) The Agency noted that the underlying EBOV infection may have confounded the assessment of signs and symptoms and their relationship with the study drug, but Section 6 would be more helpful to providers if it included the safety experience of REGN-EB3 in the context of treatment of EBOV infection. Although some data were presented in the "Findings About" datasets, these data were missing terms that were reported in some of the investigator's narratives.

In response, the Applicant resubmitted the "Findings About" analysis dataset as "adfa2.xpt," which included terms recorded on the CRF. Terms found in some of the narratives were also included either verbatim or as MedDRA-coded terms if not already reported in the CRF. The response also included an annotated CRF, which described how adverse reactions were recorded (Figure 6). "Adverse Reactions" were collected on the CRF, and additional events were reported under the category of "other adverse reactions." The Applicant provided verbatim and MedDRA-coded terms for both categories.

Figure 6. Annotated Case Report Form, Reporting of Infusion-Related Adverse Reactions

Source: Applicant's annotated case report form.

Table 25 summarizes infusion-related adverse events based on the reports in the CRF, as described above, as well as terms used in the narratives. However, it should be noted that terms from the narratives were not reported consistently because they were not specifically intended to capture infusion reactions. Narratives were provided only for subjects who died and often did not include associated information, such as assessment of their relationship to the study drug infusion. Because there were more deaths in the ZMapp arm, there were more narratives for the ZMapp arm than the REGN-EB3 arm. Due to concerns about consistency of reporting, this table was not included in the label. (b) (4)

Table 25. Adverse Events Occurring With an Incidence of ≥1% in the REGN-EB3 Arm, Safety Population, PALM Trial

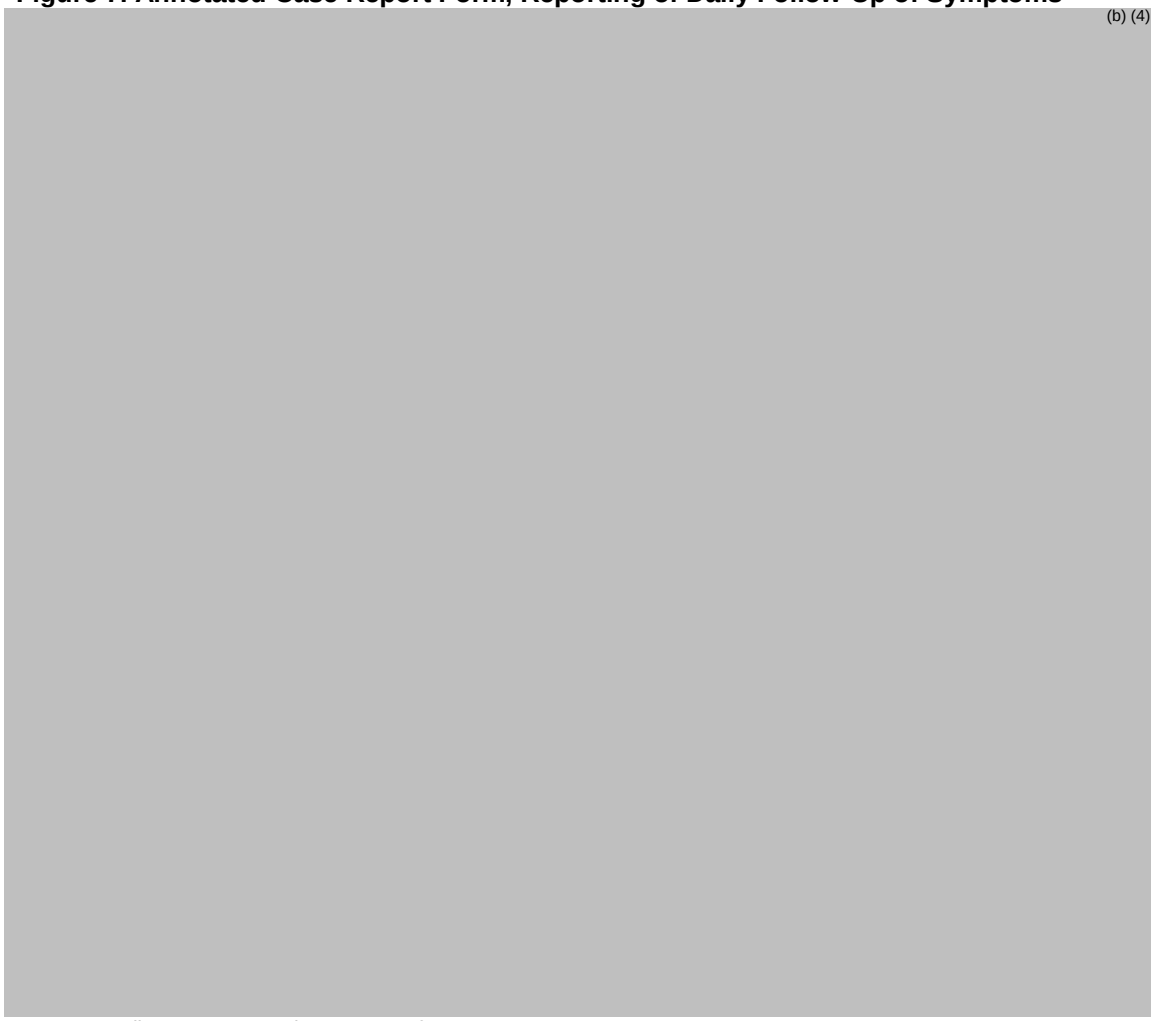
Adverse Event[a]	REGN-EB3 (N=154) n (%)	ZMapp[c] (N=168) n (%)
Any Adverse Event	120 (77.9)	151 (89.9)
Pyrexia[b] (elevation in fever)	83 (53.9)	99 (58.9)
Chills[b]	62 (40.3)	56 (33.3)
Tachycardia[b]	33 (21.4)	54 (32.1)
Tachypnea[b]	32 (20.8)	47 (28.0)
Vomiting	31 (20.1)	39 (23.2)
Hypotension[b]	24 (15.6)	52 (31.0)
Diarrhea[b]	18 (11.7)	34 (20.2)
Hypoxia	16 (10.4)	21 (12.5)

Adverse Event[a]	REGN-EB3 (N=154) n (%)	ZMapp[c] (N=168) n (%)
Hypertension[b]	12 (7.8)	17 (10.1)
Dyspnea[b]	9 (5.8)	13 (7.7)
Nausea	8 (5.2)	12 (7.1)
Asthenia	7 (4.5)	5 (3.0)
Headache	6 (3.9)	8 (4.8)
Anorexia	5 (3.2)	7 (4.2)
Cough	5 (3.2)	7 (4.2)
Agitation	4 (2.6)	8 (4.8)
Abdominal pain upper	4 (2.6)	3 (1.8)
Seizure	3 (1.9)	6 (3.6)
Pruritus[b]	3 (1.9)	2 (1.2)
Abdominal pain	3 (1.9)	7 (4.2)
Chest pain[b]	3 (1.9)	9 (5.4)
Tremor	2 (1.3)	1 (0.6)
Vertigo	2 (1.3)	5 (3.0)

Source: Reviewer's analysis of the afdas.xpt dataset using JReview. Filter was applied to "parcat3": "Adverse Reactions during Infusion coded term", "Other Adverse Reactions during Infusion coded term", and "AE Term from Death Narratives coded term." List of terms is in descending order of incidence in the REGN-EB3 arm.
[a] Adverse events in this table were reported as preferred terms from a list of predefined or other adverse events that occurred reported on the day of infusion, and included signs and symptoms that occurred during or immediately after infusion. These terms were reported in the CRF and in narratives for subject death reports. The MedDRA (version 22.0) coding dictionary was used.
[b] Adverse events that were prespecified. Note that "elevation in fever" mapped to the MedDRA term "pyrexia."
[c] Adverse events were reported on the day of infusion, ZMapp was to be administered as three separate infusions on up to three separate days.
Abbreviations: N, number of subjects; n, number of subjects with adverse event; PALM, PAmoja TuLinde Maisha

Additional signs and symptoms were also reported on a daily basis while subjects were in the ETU. The following prespecified symptoms were collected: fever, cough, mental state change, hearing loss, vision loss, headache, vomiting, diarrhea, abdominal pain, shortness of breath/difficulty breathing, hiccups, rash, edema, conjunctival injection, convulsions, hemorrhage (Figure 7). Other symptoms reported by the investigator were also included as a separate category under "Other current symptoms." Presentation of these findings help to describe safety findings beyond the immediate adverse reactions reported on the day(s) of infusion. Prespecified symptoms, however, were meant to closely follow resolution of the infection; therefore, the ability to assess the relationship of these symptoms to the study drug as "adverse drug reactions" is limited.

Figure 7. Annotated Case Report Form, Reporting of Daily Follow-Up of Symptoms



Source: Applicant's annotated case report form.

Table 26 summarizes the prespecified symptoms experienced postbaseline. There was some uncertainty with this analysis, however, due to inconsistencies found in the fa.xpt dataset when compared with the NIH datasets submitted to IND-125530. The discrepancies were not clinically significant, but an audit of the data suggested that there may have been errors in translating data that impacted the analysis study dates associated with some symptoms. Estimates of the incidence for the most common symptoms (diarrhea, fever, and vomiting) were satisfactory to include in labeling as occurring in at least 40% of subjects in the days following infusion; however, given possible errors in transposition of study dates, the exact percentage for the most common symptoms were not included in labeling.

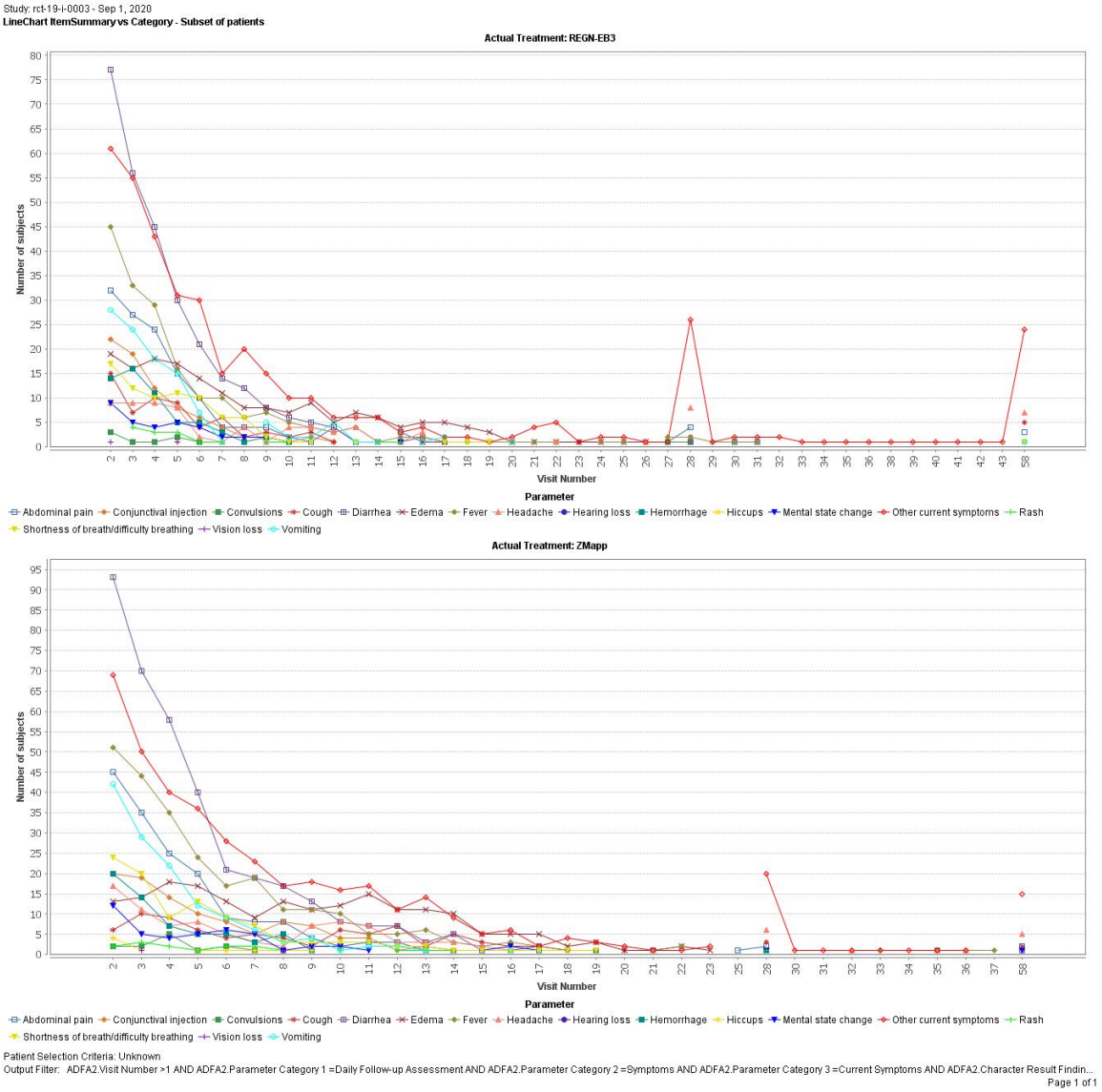
Table 26. Prespecified Symptoms Experienced Postbaseline, Safety Population, PALM Trial

Symptom	REGN-EB3 (N=154) n (%)	ZMapp (N=168) n (%)
Any symptom	123 (79.9%)	119 (70.8%)
Other symptoms (not prespecified)	109 (70.8%)	107 (63.7%)
Diarrhea	92 (59.7%)	102 (60.7%)
Fever	68 (44.2%)	70 (41.7%)
Vomiting	62 (40.3%)	63 (37.5%)
Abdominal pain	60 (39.0%)	69 (41.1%)
Headache	47 (30.5%)	45 (26.8%)
Edema	42 (27.3%)	42 (25.0%)
Conjunctival injection	37 (24.0%)	38 (22.6%)
Shortness of breath/difficulty breathing	34 (22.1%)	47 (28.0%)
Cough	33 (21.4%)	33 (19.6%)
Hemorrhage	29 (18.8%)	31 (18.5%)
Mental state change	21 (13.6%)	31 (18.5%)
Rash	11 (7.1%)	13 (7.7%)
Convulsions	6 (3.9%)	11 (6.5%)
Vision loss	2 (1.3%)	2 (1.2%)
Hiccups	1 (0.6%)	6 (3.6%)
Hearing loss	0	1 (0.6%)

Source: Reviewer analysis of adfa2.xpt using JReview. Symptoms were reported on a daily basis covering the prior 24-hour period. For this table, symptoms include the prespecified terms on the CRF on Day 2 following infusion until death or ETU discharge.
Abbreviations: N, number of subjects; n, number of subjects with adverse event; PALM, PAmoja TuLinde Maisha

Postbaseline symptoms are shown by study day and treatment arm in Figure 8. The daily incidences of the two most common symptoms in survivors decreased by approximately one half after the first and second weeks, respectively.

Figure 8. Postbaseline Symptoms by Study Day and Treatment Arm, Safety Population, PALM Trial



Source: Reviewer's analysis of adlb.xpt using JReview.
Abbreviations: PALM, PAmoja TuLinde Maisha

7.6.6. Laboratory Findings

Limited laboratory data were collected in the PALM trial in adult and pediatric subjects. Table 27 summarizes changes limited to worsening grade (using DAIDS criteria) following treatment with REGN-EB3. Laboratory tests are also reflective of the underlying illness being treated; therefore, the assessment of abnormalities is also highly confounded. For instance, there was a higher rate of mortality in the ZMapp arm compared to REGN-EB3, so relatively fewer days of laboratory data reported at similar postbaseline time points. The subgroup of subjects <18 years of age was too small to make meaningful comparisons with adult subjects.

Nevertheless, comparisons between the REGN-EB3 and ZMapp arms did not reveal significant differences in postbaseline abnormalities which may suggest a perturbation of specific laboratory parameters caused by either study drug. Similar to the trend in daily symptoms (Figure 8),

resolution of acute liver and kidney failure, or lack thereof, was driven primarily by the treatment effect of the study drug.

Table 27. Adult and Pediatric Subjects Meeting Laboratory Abnormality Criteria, Cumulative Worsened Grade From Baseline, Safety Population, PALM Trial

Laboratory Test	REGN-EB3 N=154 n (%)	ZMapp N=168 n (%)
Sodium, increased		
Any grade (≥146 mmol/L)	34 (22.1)	27 (16.1)
Grade 3 or 4 (≥154 mmol/L)	14 (9.1)	7 (4.2)
Sodium, decreased		
Any grade (<135 mmol/L)	61 (39.6)	56 (33.3)
Grade 3 or 4 (<125 mmol/L)	11 (7.1)	19 (11.3)
Potassium, increased		
Any grade (≥5.6 mmol/L)	47 (30.5)	32 (19)
Grade 3 or 4 (≥6.5 mmol/L)	20 (13)	20 (11.9)
Potassium, decreased		
Any grade (<3.4 mmol/L)	43 (27.9)	41 (24.4)
Grade 3 or 4 (<2.5 mmol/L)	14 (9.1)	13 (7.7)
Creatinine, increased		
Any grade (≥1.1× ULN[a])	31 (20.1)	42 (25)
Grade 3 or 4 (>1.8× ULN[a] or increase to ≥1.5× baseline)	23 (14.9)	39 (23.2)
Alanine aminotransferase (U/L)		
Any grade (≥1.25× ULN)	49 (31.8)	52 (31)
Grade 3 or 4 (≥5× ULN)	16 (10.4)	24 (14.3)
Aspartate aminotransferase (U/L)		
Any grade (≥1.25× ULN)	38 (24.7)	34 (20.2)
Grade 3 or 4 (≥5× ULN)	33 (21.4)	30 (17.9)

Source: ad b.xpt, Software: R
Grading scale used was DAIDS corrected version 2.1.
[a] ULN for serum creatinine=1.2 mg/dL
Abbreviations: N, number of subjects with relevant laboratory data; n, number of subjects with abnormality; PALM, PAmoja TuLinde Maisha; ULN, upper limit of normal,

Additional analyses of laboratory data from the PALM RCT are presented in Section III.17. However, clinical laboratory data were not systematically collected in EAP 1846.

7.7. Review Issues Relevant to the Evaluation of Risk

Review issues relevant to the evaluation of risk include:

- The development of resistance against REGN-EB3 has not been adequately characterized.
- Potential risks of immunogenicity.
- Risks associated with the proposed total infusion volumes and infusion times for neonates.

7.7.1. Development of Resistance Against REGN-EB3 Has Not Been Adequately Characterized

Issue

Limited resistance data were provided to identify resistance pathways for each mAb in REGN-EB3, and no clinical or relevant animal study has fully characterized the potential for clinically significant resistance substitutions associated with one or more of the individual mAbs or the potential for cross-resistance between atoltivimab (REGN3470) and odesivimab (REGN3471).

Background

Cell Culture Selection Experiments. The Applicant has not selected in cell culture virus resistant to maftivimab (REGN3479) or characterized several independent isolates genotypically and phenotypically to identify amino acid substitutions in GP that lead to reduced susceptibility. Of note, atoltivimab (REGN3470) and odesivimab (REGN3471) are not neutralizing in a live virus assay (Section III.18) and therefore characterization of treatment failure isolates is critical. Because atoltivimab (REGN3470) neutralizes VLPs, it may be possible to use pseudoviruses to select for EBOV GP variants that are less susceptible to atoltivimab (REGN3470) (Section III.18).

Overlapping Antiviral Activity of the Three mAbs in the REGN-EB3 Cocktail. The Applicant did not provide sufficient data to determine the extent to which each mAb contributes to the overall antiviral activity or if each mAb is required for the cocktail to be effective. In the event that resistance developed against one mAb in the cocktail, it is currently unknown if the other two mAbs would have sufficient antiviral activity to be efficacious. The Applicant provided two study reports describing a study performed in guinea pigs challenged with guinea pig-adapted EBOV that evaluated various combinations of the three REGN-EB3 mAbs (Section III.18). Given the limitations of this model; i.e., adaptive changes in the GP of the guinea pig-adapted EBOV that may impact susceptibility to one or more of the mAbs, and the experimental approach, it was not clear to what extent each mAb contributes to the overall antiviral activity or if each mAb is required for the cocktail to be effective. Clinical Virology's position is that the Applicant should assess the impact of atoltivimab (REGN3470)-resistance pathways on ADCC/ADCP for odesivimab (REGN3471), and the impact of maftivimab (REGN3479)-resistance substitutions on ADCC/ADCP for atoltivimab (REGN3470) and odesivimab (REGN3471). These deficiencies will be addressed in two PMRs and one PMC related to resistance (22).

Identification of the Binding Footprints for the REGN-EB3 mAbs. The epitopes of the three mAbs have not been precisely described; however, the regions of the EBOV GP that were protected during binding of the individual REGN-EB3 mAbs to GP were determined using hydrogen deuterium exchange mass spectrometry to identify putative areas of antibody-antigen contact (Section III.18). Atoltivimab (REGN3470) binds the EBOV GP parallel to the viral surface on the outside of the glycan cap, in three putative epitope segments that include residues 236 to 244, 264 to 287, and 298 to 308. These three segments span the boundary between the region shared by sGP (residues 1 to 295) and GP (residues 1 to 676) in the region unique to GP (296 to 676). However, atoltivimab (REGN3470) did not bind sGP in a Bio-Layer Interferometry study (Section III.18), indicating that the portion of the epitope in the GP region of the protein is

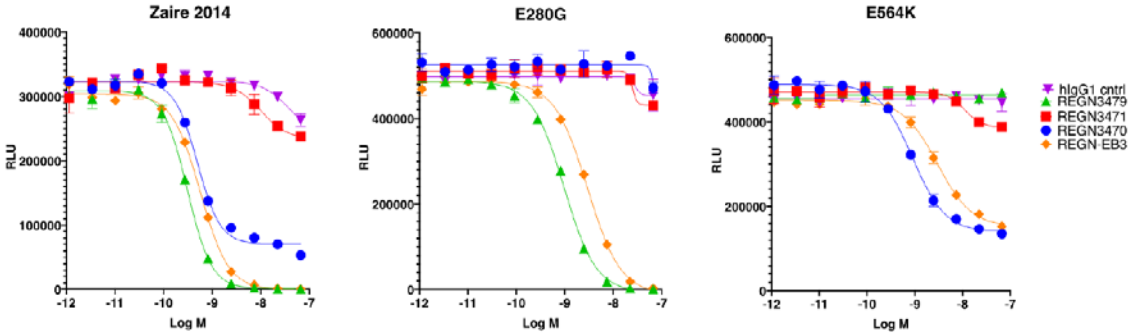
essential for binding. Odesivimab (REGN3471) binds perpendicular to the viral surface and binds to both GP and sGP in putative epitope segments that include residues 114 to 122, 139 to 151, 236 to 244, and 265 to 287. The overlap in the binding footprints of atoltivimab (REGN3470) and odesivimab (REGN3471) raises concerns about cross-resistance between these two mAbs. This will be addressed as a PMR (Section III.22). Maftivimab (REGN3479) binds at the base of GP, between protomers of GP1/GP2, with the angle of approach being slightly upward from the viral surface. The putative epitope lies near the internal fusion loop (amino acids 511 to 556) and cathepsin cleavage site (amino acids 134, 194, and 195) in one putative epitope segment that contains residues 531 to 545 (Section III.18).

Identification of GP Substitutions that Reduce REGN-EB3 Antiviral Activity. Two amino acid substitutions associated with reduced susceptibility against the REGN-EB3 mAbs have been identified and assessed using lentivirus-based VLPs pseudotyped with EBOV Makona GPs with each substitution (Figure 9). Substitution GP_E564K was identified in an infected NHP PK study (Section III.18) and substitution GP_E280G was identified by routine surveillance during the EBOV outbreak that began in the DRC in August 2018 (Mulangu et al. 2019). The GP_E564K substitution (detected in an NHP treated with 30 mg/kg REGN-EB3) knocked out the neutralization activity of maftivimab (REGN3479) and resulted in a >5-fold shift in the REGN-EB3 EC_{50} value from 0.57nM to 3.0nM (Section III.18), despite the fact that the GP_E564K substitution occurred outside of the maftivimab (REGN3479) binding region (GP amino acids 531 to 545) (Figure 9).

Substitution GP_E280G (identified by routine surveillance during the EBOV outbreak) was assessed because it lies within the atoltivimab (REGN3470) footprint and led to complete loss of atoltivimab (REGN3470) mediated neutralization (blue line) (Figure 9). Maftivimab (REGN3479) (green line) and the REGN-EB3 cocktail (orange line) retained neutralization activity against the pseudovirus, but there was a >5-fold shift in the REGN-EB3 EC_{50} value from 0.57nM to 2.9nM (Section III.18). Of note, atoltivimab (REGN3470) and odesivimab (REGN3471) have been shown to have ADCC/ADCP in cell culture assays (Section III.18). The Applicant did not assess odesivimab (REGN3471) for potential cross-resistance with atoltivimab (REGN3470) in their ADCC/ADCP assays. Assessing atoltivimab (REGN3470) and odesivimab (REGN3471) cross-resistance by ADCC/ADCP assay will be a PMR (Section III.22).

The Applicant reported that they searched 2,744 published EBOV genomes and the GP_E280G variant (or other variants at the same position) was not detected. The Applicant has not been provided any information on the source of this variant containing the GP_E208G substitution and it is not known if it developed in a subject being treated with REGN-EB3.

Figure 9. Effect of GP Sequence Variants on Virus-Like Particle Neutralization



Source: Sequence Variants Report, Figure 1, page 2
Abbreviations: GP, glycoprotein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; RLU, relative luminescence units

In addition to the GP_E564K substitution that was characterized, several additional potential resistance-associated substitutions were identified in the infected NHP PK study, including GP_E564A, GP_I544T, GP_G528R, GP_H549R, and GP_N563T. However, the Applicant did not phenotypically assess these substitutions. Phenotypic assessment of these substitutions will be a PMR (22).

Cross-Resistance Assessments in Cell Culture. In the clinical trial PREVAIL II (NCT02363322), ZMapp was used as the standard of care in protocols assessing the impact of investigational products during the EBOV outbreak that began in the DRC in August 2018 (Mulangu et al. 2019). As a result, Clinical Virology requested that the Applicant assess substitutions in GP that had been associated with escape from ZMapp mAbs (Qiu et al. 2012; Audet et al. 2014; Murin et al. 2014; Davidson et al. 2015). The substitutions of interest were GP_I274M and GP_W275L, which are in the 13c6 epitope, and GP_Q508R, which impacts mAb binding at the base of GP, 2G4, and 4G7 (Davidson et al. 2015).

The Applicant provided a study report showing assessments of GP_Q508R (Table 28); however, they did not assess GP_I274M and GP_W275L. The binding footprint for maftivimab (REGN3479) corresponding to GP residues 531 to 545 is distinct from the Q508 position associated with resistance to ZMapp. Of note, GP_I274M and GP_W275L fall within the binding footprints of both atoltivimab (REGN3470) and odesivimab (REGN3471), but these substitutions were not assessed for their impact on REGN-EB3 or atoltivimab (REGN3470) and odesivimab (REGN3471). Determining the phenotypes of these substitutions is included in a PMR (22).

The GP_Q508R substitution has been shown to impact other mAbs binding at the base (KZ52, 2G4, and 4G7; (Davidson et al. 2015). The anti-EBOV GP mAbs were further tested for cell culture neutralization of VLPs generated with the EBOV Kikwit 1995 GP containing the ZMapp GP_Q508R escape substitution. Atoltivimab (REGN3470) and maftivimab (REGN3479) neutralized VLPs pseudotyped with EBOV Kikwit 1995-GP_Q508R GP with EC_{50} values of 0.19nM and 0.16nM, respectively (Table 28). These EC_{50} values are similar to those determined based on neutralization of EBOV Makona 2014 VLPs (Table 28). Odesivimab (REGN3471) displayed very weak EBOV Kikwit 1995 VLP neutralization, similar to that observed for neutralization of EBOV Makona 2014 VLPs. The REGN-EB3 cocktail neutralized EBOV Kikwit 1995 VLPs with an EC_{50} value of 0.38nM (Table 28). These results indicate that the GP_Q508R substitution does not reduce susceptibility (<2-fold shift in EC_{50} value) to

maftivimab (REGN3479), which is consistent with the hydrogen deuterium exchange mass spectrometry results described above.

Table 28. EC_{50} Values for Cell Culture Neutralization of EBOV VLPs With REGN-EB3 mAbs

	EC_{50} value (M)		
Antibody	**Makona 2014**	**Kikwit 1995-Q508R**	**Fold shift**
Atoltivimab (REGN3470)	2.00E-10	1.94E-10	1.0
Odesivimab (REGN3471)	4.49E-08	1.08E-08	0.2
Maftivimab (REGN3479)	8.28E-11	1.59E-10	1.9
REGN-EB3	2.84E-10	3.76E-10	1.3

Source: Modified from Table 2, page 13, REGN3479-MX-16084-SR-01V1
Abbreviations: EBOV, *Zaire ebolavirus*, EC_{50}, half-maximal effective concentration, mAbs, monoclonal antibodies; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; VLPs, virus-like particles

The GP_Q508R substitution associated with resistance to ZMapp did not confer cross-resistance to REGN-EB3 or any of its constituent mAbs. However, REGN-EB3 may select for resistant EBOV variants that are also resistant to other mAbs being developed against EBOV.

Assessment of resistance in subjects treated with REGN-EB3 in the PALM clinical trial. No resistance data from the PALM clinical trial have been provided. A PMC has been agreed upon with the Applicant to assess REGN-EB3 resistance in samples collected in the PALM trial if those data become available from the sponsor of that trial (Section III.22).

Assessment

The Clinical Virology reviewer reviewed the totality of the resistance data provided by the Applicant and concluded that the data provided were insufficient to adequately characterize resistance to the REGN-EB3 cocktail or the individual mAbs contained therein. The review team was notified of the incomplete characterization of resistance, and it was agreed that additional resistance data would be requested as PMRs and PMCs (Section III.22).

Conclusion

The incomplete characterization of resistance described in this section will be addressed by two PMRs related to further characterizing resistance to REGN-EB3 mAbs and cross-resistance between atoltivimab (REGN3470) and odesivimab (REGN3471) by ADCC/ADCP assay. In addition, the Applicant has agreed to a PMC to precisely define the epitopes of each mAb. A second PMC has been agreed upon with the Applicant to assess REGN-EB3 resistance in samples collected in the PALM trial if those data become available from the sponsor of that trial. Additional details are provided in Section III.22.

7.7.2. Potential Risks of Immunogenicity

Issue

Immune responses to therapeutic protein products may pose problems for both patient safety and product efficacy. The major safety concerns associated with immunogenicity include anaphylaxis, hypersensitivity, and other infusion-related reactions.

Background

An immunogenicity study that is designed to assess whether ADAs are induced by a first dose, and potentially associated with important safety findings of a second dose, was considered as a potential PMR. The study may be able to address the implications for repeat exposure as well as

inform potential therapeutic options for mitigation of infusion reactions. The presence of ADAs alone, however, is not necessarily predictive of anaphylaxis or other hypersensitivity reactions.

Assessment

No ADAs, or anti-atoltivimab, anti-maftivimab, or anti-odesivimab antibodies were detected in the first-in-human, healthy volunteer study (Study 1528) following a single dose of REGN-EB3. This study, however, did not use validated methods, including assays for detection of neutralizing antibodies. Assays for ADAs were also not available for any EBOV-infected subjects treated in either the PALM trial or in EAP 1846.

REGN-EB3 was only studied in clinical trials involving a single infusion; however, there may be circumstances under which prescribers would consider a second dose, such as late recurrence due to chronic persistence in immune-privileged sites (e.g., uveitis, orchitis), or reinfection. No such cases, however, have been described following treatment with REGN-EB3.

Conclusion

The review team declined to further pursue a PMR to further study immunogenicity, because the recommended dosing regimen for the proposed indication is only a single infusion. The likelihood of a second dose was also considered low, because there were no cases of late recurrence or reinfection after treatment with REGN-EB3 in the PALM RCT or EAP 1846. (b) (4)

7.7.3. Risks Associated With the Proposed Total Infusion Volumes and Infusion Times for Neonates

Issue

The proposed Dosage and Administration section of the label instructed users to dilute INMAZEB in 0.9% sodium chloride injection, 5% dextrose injection, or lactated ringer's injection. Potential risks include compatibility issues when coadministering with other infusions, endotoxin levels exceeding the recommended limit when REGN-EB3 is combined with large volumes of diluents, volume overload, electrolyte imbalance, and inadequate nutritional supplementation.

Background

The primary review division (DAV) placed an interoffice neonatal-perinatal medicine consultation request to the Office of Pediatric Therapeutics (OPT). In the responding memorandum, Gerri Baer, MD, Supervisory Medical Officer for the Pharmacovigilance and Neonatology Team, noted that in preterm neonates, especially those of <2 kg birth weight, balancing fluid, and electrolyte status to avoid generalized edema/anasarca, pulmonary edema, patent ductus arteriosus, and intraventricular hemorrhage is critical. In addition, the glomerular filtration rate is low at birth and increases over the first year of life, with otherwise healthy preterm neonates having a glomerular filtration rate as low as 10 to 20 mL/min/1.73m^2 at birth (Kastl 2017). The daily fluid intake for extremely preterm neonates is typically maintained at 140 to 180 mL/kg/day, with higher fluid intakes needed at times by neonates with significant insensible losses. For example, a 0.5 kg neonate may require up to 200 mL/kg/day.

Note that the of the memorandum from OPT are also reflected in the assessment and conclusions of this section.

Assessment

Based on the initial clinical experience using the desired 2-hour infusion time, the Applicant's proposed infusion volumes recommended a final concentration of diluted solution of 9.5 to 23.7 mg/mL. Table 29 summarizes theoretical infusion volumes and rates for patients weighing <2 kg based on acceptable levels of endotoxin input when combined with 5% Dextrose Injection, USP. Under 2 kg, the initial volumes for administration were not optimal. A medication volume of 20 mL in a 0.5 kg neonate is about 25% of their daily fluid intake.

Table 29. Theoretical Infusion Volumes and Infusion Rates of INMAZEB for Patients Weighing Less Than 2 kg

Patient Weight (kg)	Hourly Total Fluid Target (mL/hr)[a]	Protein Concentration (mg/mL)	Infusion Time (hours)	Infusion Volume (mL)	Fluid Volume per Hour (mL/hr)	Total Endotoxin Input (EU/kg/hr)
0.5	2.9-4.2	3.75	3	20	6.7	7.17[b]
0.5	2.9-4.2	3.75	4	20	5	5.38[b]
0.5	2.9-4.2	5	3	15	5	5.50[b]
0.5	2.9-4.2	5	3.5	15	4.3	4.71
0.5	2.9-4.2	5	4	15	3.8	4.13
1.0	5.8-7.5	6	3	25	8.3	4.67
1.5	8.8-11.3	9	3	25	8.3	3.28
2.0	11.7-15	12	3	25	8.3	2.58

Source: Reviewer's analysis. [a] Target based on total daily fluid of 140-200 mL/kg/day for a 0.5 kg neonate, or up to 180 mL/kg/day in neonates ≥1.0 kg. [b] Endotoxin input level (b) (4)

The Applicant's proposed modifications (Table 30) satisfied the concerns about fluid volume and overload in neonates weighing <2 kg.

Table 30. INMAZEB Infusion Volumes and Times by Body Weight

Body Weight (kg)	Volume of INMAZEB per kg of Body Weight[a]	Prepared Infusion Volume (mL)[b]	Infusion Time
0.5 to less than 1	3 mL per kg of body weight	7	
1 to 1.9		15	4 hours
2 to 3.9		25	
4 to 7		50	
8 to 15		100	3 hours
16 to 38		250	
39 to 79		500	
80 to 149		1,000	2 hours
150 and above		2,000	4 hours

[a] The dose is 50 mg of atoltivimab, 50 mg of maftivimab, and 50 mg of odesivimab per kg body weight (a volume of 3 mL/kg).
[b] The recommended infusion volume ensures the final concentration of the diluted solution is 9.5 mg/mL to 23.7 mg/mL. 5% Dextrose Injection, USP is recommended for neonates.

Addition of the following text will also be added to the label under the heading "Preparation for Intravenous Infusion": "5% Dextrose Injection, USP is recommended for neonates."

Because no compatibility testing was performed, ideally, INMAZEB would be administered by itself in a separate infusion line. However, multiple IV sites may not be available in critically ill

neonates, which may necessitate coadministration or administration in adjacent lumens of a central line.

EBOV-infected neonates would likely need some IV nutrition support, at a minimum, IV dextrose, so that they do not become hypoglycemic during the infusion. This population of neonates may also require additional medications for life support, including vasopressors. In the critically ill neonate, if multiple IV sites are not available, clinicians could use their discretion in the administration of multiple life-saving medications.

Conclusion

To address the unique fluid challenges in premature neonates, the following modifications to Section 2.2 Preparation and Administration of the proposed label have been made:

1. Amendments that provide acceptable infusion volumes and rates for neonates <2 kg.
2. Labeling should include a footnote to the "Infusion Volumes and Times by Body Weight" table with the accompanying recommendation for dilution with 5% Dextrose Injection, USP with neonates.
3. Coadministration may be required in emergent circumstances; therefore, the proposed language in labeling should explain that compatibility testing was not performed, (b) (4)

8. Therapeutic Individualization

8.1. Intrinsic Factors

PK was not evaluated in EBOV-infected subjects in the PALM trial. The PK of REGN-EB3 were evaluated in healthy adults aged 21 to 60 years with a normal BMI and normal renal and hepatic function. The impact of age (pediatric or geriatric), obesity, organ impairment (renal or hepatic impairment) or pregnancy on the PK of REGN-EB3 has not been evaluated. The PK of therapeutic proteins (>40 kDa) such as REGN-EB3 is not expected to be altered in patients with renal impairment. Limited information is available on the potential impact of hepatic impairment on the PK of mAbs. We recommend evaluating the PK of REGN-EB3 in infected subjects in future clinical trials. This will enable evaluation of exposure-response relationships for efficacy and safety as well as the impact of demographic factors on PK.

8.2. Drug Interactions

Enzyme- or Transporter-Mediated Interactions

DDI studies (in vitro or in vivo) were not conducted. Because REGN-EB3 consists of mAbs targeting an exogenous viral protein, it is not expected to be a victim or perpetrator of drug metabolizing enzyme- or transporter-mediated interactions.

Vaccine Interactions

No vaccine-therapeutic interaction studies have been performed in human subjects using REGN-EB3. The efficacy of REGN-EB3 among subjects who reported receipt of a recombinant live vaccine prior to their enrollment in the PALM clinical trial was similar to that of subjects who did not receive vaccine. However, because of the potential for REGN-EB3 to inhibit replication

of a live vaccine virus indicated for prevention of EBOV infection and possibly reduce the efficacy of the vaccine, the labeling states to avoid the concurrent administration of a live vaccine during treatment with REGN-EB3.

8.3. Pediatric Labeling / Plans for Pediatric Drug Development

REGN-EB3 was granted Orphan Drug Designation (#16-5363) for the treatment of patients with EBOV infection on July 14, 2016. With this designation, it was exempted from the PREA requirements. Nevertheless, adequate clinical experience was provided to evaluate the benefit and potential risks in all pediatric populations, including low-birth-weight neonates born to EBOV-infected mothers. The review issue relevant to the evaluation of benefit for pediatric populations is discussed in Section II.6.4.4. The review issue relevant to the evaluation of risk associated with the proposed total volumes for infusion and the proposed total infusion volumes and infusion times for neonates is discussed in Section II.7.7.3.

8.4. Pregnancy and Lactation

Pregnancy

REGN-EB3 is directed against an exogenous target (EBOV GP); therefore, no reproductive or developmental toxicology studies were performed. There were no findings in the repeated-dose toxicology study or off-target tissue binding of the mAb(s) that were predicted to impact reproduction. Based on this limited nonclinical assessment of reproductive/developmental risk, REGN-EB3 is not anticipated to affect pregnancy or lactation.

Available data from the 16 pregnancies identified during the PALM RCT (n=3), PALM-Extension Phase (n=5), and EAP (n=8) for REGN-EB3 are insufficient to evaluate a drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes (Table 31, Table 32, and Table 33). The high rates of maternal and fetal/neonatal morbidity and mortality observed in these studies are consistent with the published literature regarding the risks to pregnancy associated with underlying maternal EBOV infection. Because EBOV is life-threatening for both the mother and fetus, treatment should not be withheld due to pregnancy.

For Pregnancy and Lactation Labeling Rule (PLLR) labeling, the Risk Summary in Subsection 8.1 Pregnancy will reflect the above conclusions. The PLLR background risk statement will be omitted because it may be misleading considering that the rate of miscarriage in patients infected with EBOV is much higher than the reported 15 to 20% in the U.S. general population. The indication-specific background risk statement will also be omitted because it is inapplicable considering that infection with EBOV is life-threatening for both the mother and fetus, and treatment should not be withheld due to pregnancy. In addition, a Clinical Consideration will be included that maternal, fetal, and neonatal outcomes are poor among pregnant women infected with EBOV, with the majority of such pregnancies resulting in maternal death with miscarriage, stillbirth, or neonatal death. Thus, treatment should not be withheld due to pregnancy. Finally, because REGN-EB3 is a combination of three human IgG1 mAbs, the labeling will include a statement that mAbs cross the placenta to the fetus.

Table 31. Pregnancy Outcomes Following Exposure to RGN-EB3 During PALM RCT

Subject ID	Maternal Age/Gravidity and Parity	Drug Exposure	Timing of Exposure	Maternal Outcome	Fetal Outcome
(b) (6)	29 years G3P2	REGN-EB3	Second trimester	Alive at Day 58 follow-up	Livebirth at 42 weeks (128 days after treatment); no congenital anomalies Placenta, umbilical cord, amniotic fluid, and salivary swab+ for Ebola by RT-PCR Newborn randomized to another investigational product (mAb114) on the day of birth. Ebola RT-PCR on the infant blood sample was negative 1 day after treatment with mAb114.
	17 years G/P not reported	REGN-EB3	Unknown	Death on Day 2 of treatment	Unknown The subject presented with heavy vaginal bleeding prior to treatment. No pregnancy information was available.
	23 years G4P3	ZMapp on Day 1 and Day 8, then REGN-EB3 on Day 8	First trimester	Alive at Day 58 follow-up	Fetal death in utero at 16 weeks (day 96 after treatment. Event reported as a nontreatment-emergent serious adverse event.

Abbreviations: mAb, monoclonal antibody; PALM, PAmoja TuLinde Maisha; RCT, randomized controlled trial; RT-PCR, reverse transcription-polymerase chain reaction

Table 32. Pregnancy Outcomes Following Exposure to REGN-EB3 During the PALM-Extension

Subject ID	Maternal Age/ Gravidity and Parity	Drug Exposure	Timing of Exposure	Maternal Outcome[1]	Fetal Outcome
(b) (6)	30 years G6P5	REGN-EB3	Second trimester (27 weeks)	Unknown	Pregnancy terminated due to fetal distress (Day 3). Fetus with second degree maceration reported. However, the timing of the fetal loss in this case is unclear. Published literature suggest that assessing the degree of fetal maceration is not a reliable predictor of the amount of time before a fetal death occurred.
	35 years G5P4	REGN-EB3	Second trimester	Unknown	Premature birth at 29 weeks (Day 13). Neonatal death 30 min after birth despite resuscitation. Weight: 1,200 g, Apgar 3/2. No congenital anomalies.

Subject ID	Maternal Age/ Gravidity and Parity	Drug Exposure	Timing of Exposure	Maternal Outcome[1]	Fetal Outcome
(b) (6)	23 years G1P0	REGN-EB3	Third trimester (29 weeks)	Unknown	Term livebirth at 39 weeks (Day 69). Weight: 2,900 g, Apgar 9/10. No congenital anomalies. PCR of breast milk and placenta + for EVD, but maternal blood, umbilical cord, and newborns blood negative.
	28 years G3P2	REGN-EB3	First trimester (9 weeks)	Unknown	Pregnancy terminated (Day 3)
	40 years G6P5	REGN-EB3	Third trimester (35 weeks)	Unknown	Term livebirth at 40 weeks (Day 44). Newborn small for gestational age, Apgar 9/8. No congenital anomalies. Neonatal death at 48 hours after delivery due to respiratory distress.

[1] Per the NIAID, mortality data from the PALM extension phase will not be shared with any third party until completion of the study. Thus, maternal outcomes were not available in the Applicant's 60-day safety-update report.
Abbreviations: EVD, *Zaire ebolavirus* disease; PALM, PAmoja TuLinde Maisha; PCR, polymerase chain reaction

Table 33. Pregnancy Outcomes Following Exposure to REGN-EB3 Through EAP 1846

Subject ID	Maternal Age	Drug Exposure	Timing of Exposure	Maternal Outcome*	Fetal Outcome
(b) (6)	22 years	REGN-EB3	First trimester (3 months)	Alive and discharged at Day 20	Spontaneous abortion treated with curettage on Day 2. The subject presented with vaginal bleeding which suggests the miscarriage may have been in progress prior to treatment.
	20 years	REGN-EB3	First trimester (3 months)	Death on Day 1 of treatment	Spontaneous abortion reported within 24 hours before maternal death, manually evacuated.
	25 years	REGN-EB3	First trimester (11 weeks)	Death on Day 2 of treatment	Spontaneous abortion prior to treatment.
	20 years	REGN-EB3	First trimester (10 weeks)	Alive and discharged at Day 16	Unknown
	23 years	REGN-EB3	Unknown	Death on Day 5 of treatment	Unknown
	23 years	REGN-EB3	First trimester (2 months)	Alive and discharged Day 17	Unknown
	18 years	REGN-EB3	Second trimester (7 months)	Death on Day 2 of treatment	Intrauterine fetal demise (occurred prior to treatment)
	20 years	REGN-EB3	First trimester (3 months)	Death on Day 3 of treatment	Intrauterine fetal demise (occurred post-treatment; uterine evacuation performed on unknown date)

Abbreviations: EAP, expanded access protocol

Lactation

There are no available data on the presence of REGN-EB3 in human or animal milk, the effects on the breastfed infant, or the effects on milk production. The effects of local gastrointestinal exposure and limited systemic exposure in the breastfed infant to atoltivimab, maftivimab, or odesivimab are unknown. Both the Centers of Disease Control and the WHO recommend that women with EVD not breastfeed due to the reported presence of Ebola virus in breast milk and the potential for postnatal transmission in the breastfeed infant.

For PLLR labeling, the Risk Summary in Subsection 8.2 Lactation will reflect the above conclusions and include a statement that the Centers of Disease Control recommends that mothers infected with EBOV not breastfeed their infants to the reduce the risk of postnatal transmission of EBOV.

9. Product Quality

Approval with a PMC - The Office of Pharmaceutical Quality Review team has assessed BLA 761169 with respect to Chemistry, Manufacturing, and Controls (CMC) and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports. As such, OPQ recommends approval of this BLA from a quality perspective. The CMC postmarketing commitments (PMC) between OPQ and the Applicant are listed below should be included in the action letter:

Table 34: Chemistry, Manufacturing, and Controls Postmarketing Commitments

CMC PMC	Milestone
1.Re-evaluate and update REGN-EB3 drug substance (DS) and drug product lot release and stability specifications based on lots manufactured by the (b) (4) DS processes. The corresponding data, the analysis and updated specifications will be submitted with the PAS for the registration of the (b) (4) commercial manufacturing process.	Final Report Submission: 01/2021
2. Conduct a real-world Transport Qualification study that includes a product quality assessment using REGN-EB3 drug product. The real-world Transport Qualification study results will be submitted in a final report to the BLA.	Final Report Submission: 08/2021
3.Re-evaluate and optimize the manufacture, qualification and stability controls used to ensure the performance of Ebola Virus-Like Particles (VLPs) in the pseudovirus neutralization assays. The re-evaluation, development study results and the final control strategy for Ebola VLPs will be provided in the final report to the BLA.	Final Report Submission: 12/2021
4. Provide microbial hold time data in a microbial challenge study to support the total in-use time (storage and infusion time) of diluted INMAZEB in 5 % Dextrose Injection beyond 4-hours at ambient temperature. The study should be conducted for twice the worst-case in-use time and bracketing the drug product concentrations that would be administered to patients. The study should also be representative of the in-use conditions; for example, neonates may be kept at temperatures above 20-25°C during infusion and the higher temperatures should be simulated in the study supporting in-use conditions.	Final Report Submission: 04/2021

9.1. Device or Combination Product Considerations

This section is not applicable, because REGN-EB3 does not involve components that would normally be regulated under different types of regulatory authorities.

10. Human Subjects Protections / Clinical Site and Other GCP Inspections / Financial

Four Ebola Treatment Units (ETU), Beni, Katwa, Mangina, and Butembo, and the study sponsor, the National Institute of Allergy and Infectious Disease (NIAID), were inspected in support of BLA 761169. The PALM trial (Protocol 19-I-0003) had nine clinical investigators who rotated through, staffed, and supervised the conduct of the study for the 4 ETUs. Four clinical investigators were selected to represent the four ETUs during the inspections to answer questions.

Table 35. Study Sites Requested for Inspection

Name	Location	Notes
Jean-Luc Biampata, MD	Beni, DRC	337 subjects were screened, 335 were randomized [REGN-EB3 (n=72)], and 196 subjects completed the study
Ali Dilu, MD	Katwa, DRC	46 subjects were screened, 46 were randomized [REGN-EB3 (n=10)], and 27 subjects completed the study
Isekusu Mpinda Fiston, MD	Mangina, DRC	57 subjects were screened, 57 were randomized [REGN-EB3 (n=14)] and 14 subjects completed to the study
Vicky Malengera, MD	Butembo, DRC	244 subjects were screened, 243 were randomized [REGN-EB3 (n=63)] and 70 subjects completed the study
National Institute of Allergy and Infectious Disease (NIAID)	Bethesda, MD USA	Responsible for control, oversight, and management of Protocol 19-I-0003. NIAID contracted with Leidos Biomedical Research, Inc. for clinical trial management, regulatory documentation, data management. Documentation relied on the PALM Study Website, the (b) (4) Database, and the REDCap electronic data capture system.

OSI concluded that the PALM trial was conducted adequately, and the study data submitted, including the primary efficacy endpoint data, appear acceptable in support of the respective indication. Please refer to Section III.20 for the Clinical Inspection Summary from OSI.

11. Advisory Committee Summary

This application was not taken to an FDA advisory committee because the application did not raise significant safety or efficacy issues that were unexpected and there were no controversial issues that would benefit from discussion by an advisory committee.

III. Appendices

12. Summary of Regulatory History

On February 25, 2020 Regeneron Pharmaceuticals Inc. (Regeneron) submitted an original new molecular entity biologics license application (BLA) first in therapy for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479), as a combination of three human IgG1 monoclonal antibodies (mAbs) (REGN-EB3) directed against different, nonoverlapping epitopes on *Zaire ebolavirus* (EBOV) glycoprotein (GP) for the treatment of EBOV infection.

Regeneron Pharmaceuticals initiated the REGN-EB3 development program under the Animal Rule. However, with the occurrence of the Ebola outbreak in the North Kivu province of the Democratic Republic of the Congo (DRC) in 2018, the Applicant was able to obtain randomized, controlled clinical data to support the safety and effectiveness of REGN-EB3 for EBOV infection through a traditional approval pathway. The registrational investigational new drug application (IND) is IND 125507.

The application is supported by the Phase 2/3 randomized controlled trial (National Institute of Allergy and Infectious Diseases Protocol 19-I-0003, PAmoja TuLinde Maisha [PALM] Study) designed to study the comparative safety and efficacy of investigational therapeutics compared to an investigational control (ZMapp) in subjects with known EBOV disease. All subjects received optimized standard of care (oSOC). Data from the expanded-access program, R3470-3471-3479-EBOV-1846 (EAP 1846) were submitted as supportive data. The Applicant submitted requests for both the Tropical Disease and Medical Countermeasure Priority Review Vouchers. The Applicant intends to distribute REGN-EB3 to the Strategic National Stockpile postapproval.

Notable regulatory milestones for this application include:

- Orphan Drug designation (#16-5363) for REGN-EB3 for the treatment of patients with EBOV infection was granted on July 14, 2016.
- Breakthrough Therapy designation for REGN-EB3 for the treatment of EBOV infection was granted on September 3, 2019.
- Rolling review was granted on October 2, 2019.

Milestone meetings related to the request for marketing approval:

- Type C chemistry, manufacturing, and controls (CMC)-only meeting on September 4, 2019 to obtain feedback on the content of the planned BLA submission.
- Type B pre-BLA meeting was held on October 21, 2019 and agreement was reached on the content, structure, and format of the clinical portion of the BLA and the acceptance of clinical virology resistance data and description of the HDX-mapped as a 60-day late component.
- Type B pre-BLA CMC-only meeting was held on October 31, 2019 and agreement was made on the content of the CMC portion of the BLA.

Milestone meetings related to the development program of REGN-EB3:

- Type B pre-IND written request only meeting on April 3, 2015 to gain feedback on CMC, preclinical, clinical pharmacology, toxicology, and the clinical virology development program.
- Type C Animal Rule Meeting on September 16, 2016 to gain feedback on the preclinical and clinical package.
- Type C written request only toxicology meeting to gain feedback on the preclinical development program.

13. Pharmacology Toxicology Assessments and Additional Information

13.1. Summary Review of Studies Submitted Under IND

Atoltivimab, maftivimab, and odesivimab-ebgn (REGN-EB3; REGN3470, REGN3471, and REGN3479) initiated under IND 125507 as a combination of three EBOV GP-directed mAbs for the treatment of EBOV disease (EVD). All nonclinical safety studies conducted in support of REGN-EB3 were submitted as components of the BLA and are reviewed in the following sections.

13.1.1. Pharmacology (Primary and Secondary)

REGN-EB3 is a cocktail of three IgG1 antibodies (REGN3470-3471-3479), each of which targets unique noncompeting epitopes on the EBOV GP. The therapeutic approach of the cocktail is to neutralize the virus directly and eliminate EBOV-infected cells through an antibody dependent cell-mediated cytotoxicity (ADCC)-based mechanism. REGN3479 had the broadest neutralizing capability and REGN3470 and REGN3471 have shown ADCC activity in vitro. See the virology review for additional details on all cell-based activity studies.

In vivo activity was assessed via a guinea pig model and a series of NHP studies using rhesus macaques (Table 36). None of these studies was performed in a well-characterized model of Ebola disease. Because these models were exploratory, these studies are able to assess only the potential activity of REGN-EB3 in animals inoculated with a specific strain (guinea pig-adapted Mayinga strain in guinea pigs, and human Kikwit strain in NHP) of EBOV.

Table 36. Summary of Nonhuman Primate Studies Evaluating the Activity of REGN-EB3

NHP Study #	Study Duration (Days)	Treatment		Survival	
		Dosage (via IV Route)	Dosing Schedule (Days Post Infection)	Percentage (%)	Number of Surviving Animals/ n
1 (AP-14-017 IV / R3479-MX-15044)	28	3 x 50 mg/kg REGN-EB3	5, 8, 11	80	4/5
		Saline	5, 8, 11	25	1/4
2 [a] (AP-14-017 VI)	28	3 x 50 mg/kg REGN-EB3	5, 8, 11	100	9/9
		Saline	5, 8, 11	0	0/6
3 ((b) (4) 2015-002)	28	3 x 50 mg/kg REGN-EB3	5, 8, 11	75	3/4
		2 x 50 mg/kg REGN-EB3	5, 8	100	5/5
		1 x 150 mg/kg REGN-EB3	5	80	4/5
		Saline	5, 8, 11	0	0/4
4[b] (AP-14-017 IX and X)	35 to 36	1 x 150 mg/kg REGN-EB3	5	89	8/9
		1 x 100 mg/kg REGN-EB3	5	89	8/9
		1 x 50 mg/kg REGN-EB3	5	78	7/9
		1 x 10 mg/kg REGN-EB3	5	44	4/9
		Saline	5	0	0/4
5 ((b) (4) 2018-008)	47 to 49	1 x 300 mg/kg REGN-EB3	5	100	5/5
		1 x 150 mg/kg REGN-EB3	5	50	2/4
		1 x 100 mg/kg REGN-EB3	5	20	1/5
		1 x 30 mg/kg REGN-EB3	5	60	3/5
		Saline	5	0	0/4

[a] (b) (4)

[b] Due to the large size, this study was performed in 2 parts (IX and X), and viremia was monitored for an extended period of time (day 119) for part X of the study.

IV, Intravenous.

Source: 2.6.2 Pharmacology Written Summary, Table 2

Abbreviations: IV, intravenous; n, number of subjects in subgroup; NHP, nonhuman primate

Summary of Exploratory In Vivo Animal Models of EBOV Infection

Cocktails of mAbs at a 1:1:1 ratio (5 mg) administered intraperitoneally to animals 3 days postinfection prolonged survival of infected guinea pigs. In the NHPs, a more clinically relevant animal model, the cocktail was evaluated in five exploratory studies using rhesus macaques. The initial three studies showed that the antibody cocktail has activity against EBOV infection when administered as three doses of 50 mg/kg at days 5, 8 and 11; two doses of 50 mg/kg at days 5 and 8; or as a single dose of 150 mg/kg at Day 5 postinfection. The fourth study attempted to determine the minimum efficacious dose of the cocktail; 100 mg/kg was the lowest dose tested that offered ~90% survival in this NHP model of EBOV infection. The fifth NHP study (reviewed fully in the Pharmacokinetics subsection) was designed to determine the (PK/PD) relationship of the individual mAbs following REGN-EB3 dosing. All treatment groups demonstrated improved survival compared to the placebo group. However, the small number of animals per group in combination with the skewed distribution of viral load and secreted glycoprotein (sGP) across groups makes it difficult to draw conclusions on activity across the dose groups assessed. In general, all of the in vivo studies performed had limitations that hamper development of an adequate dose-response relationship. See the virology review for additional details on in vivo activity studies.

13.1.2. Safety Pharmacology

Safety pharmacology endpoints were integrated into the good laboratory practices (GLP)-compliant repeat-dose toxicology study conducted in Sprague-Dawley rats (See General Toxicology study). There were no test article-related effects on the cardiovascular (heart rate and blood pressure evaluated via telemetry) or central nervous (functional observational battery) systems when the cocktail was administered at doses up to 195 mg/kg (administered intravenously [IV] at a 1:1:1 ratio; 65 mg/kg/antibody) once weekly for 3 consecutive weeks.

13.1.3. Pharmacokinetics

The pharmacokinetics (PK)/toxicokinetics of REGN-EB3 were characterized following a single-dose IV PK study in cynomolgus macaques and a repeat-dose IV toxicology study in Sprague-Dawley rats. Additionally, to assess the impact of EBOV infection on PK, a single-dose IV PK study in uninfected rhesus macaques and a single-dose IV PK/ pharmacodynamics (PD) study in EBOV-infected rhesus macaques were conducted. In all studies, REGN-EB3 was administered at a 1:1:1 ratio of the three mAbs; REGN-EB3 dose levels represent the total dose of the three mAbs. Because REGN-EB3 is a mAb cocktail that targets an exogenous protein, EBOV GP, ADME studies are not warranted. The profile of each individual mAb following administration of REGN-EB3 in multiple models is described.

PK in Cynomolgus Macaque

REGN3479, REGN3471, and REGN3470: A Single-Dose Intravenous Infusion PK Study in Cynomolgus Monkeys (Study #REGN3479-PK-15086)

Table 37. PK in Uninfected Cynomolgus Macaque Study Design

Methods	Details
Testing facility:	(b) (4)
Dose and frequency of dosing:	See experimental design below; single 30-minute infusion
Route of administration:	Intravenous

Methods	Details
Formulation/vehicle:	Vehicle: Saline
Species/strain:	Cynomolgus macaque
Number/sex/group:	See experimental design below
Age:	3.2 to 4.7 years
Satellite groups/unique design:	See experimental design below
Deviations affecting interpretation:	None

Abbreviations: NV, Nevada; PK, pharmacokinetics

Table 38. Experimental Design of PK Study in Uninfected Cynomolgus Macaque

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals[a]	
					Males	Females
1	REGN3479	17.0 [b]	1	17.0	3	3
2	REGN3471	16.2 [b]	1	16.2	3	3
3	REGN3470	16.7 [b]	1	16.7	3	3
4[c]	REGN3479/REGN3471/ REGN3470	5 mg total (~1.7 per mAb)	1	5	3	-
		50 mg total (~16.7 per mAb)	1	50	-	3
5[c]	REGN3479/REGN3471/ REGN3470	50 mg total (~16.7 per mAb)	1	50	3	-
		5 mg total (~1.7 per mAb)	1	5	-	3

mAb = monoclonal antibody; - = not applicable.
[a] All animals were returned to colony on Day 85.
[b] Dose levels for Groups 1, 2 and 3 were based on the actual concentrations of test articles.
[c] Based on the bioanalytical data provided by the Sponsor and internal investigation conducted by the Testing Facility, it was surmised that the Group 4 females were given Group 5 dosing material and that the Group 5 females were given Group 4 dosing material.

Source: eCtd loc. 4.2.2.2
Abbreviations: PK, pharmacokinetics; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

To determine the PK characteristics of REGN-EB3 and each of its components, cynomolgus macaques were intravenously infused with the combination (5 and 50 mg/kg) or each individual mAb (17, 16.2, and 16.7 mg/kg, respectively). There was an error in dosing, which was investigated by the Testing Facility. The investigation traced the error to one of two possibilities: either incorrect dose material was withdrawn into the Group-4 and -5 female syringes in the formulations laboratory or the material was properly withdrawn, but the animals were dosed with the incorrect syringes. Because this was a single-dose study, the study results were interpreted based on this assumption. The study did not have concurrent controls. The precipitous decline in the REGN3479, REGN3471, or REGN3470 concentration observed in 4 of the 30 (13%) animals was consistent with an antidrug antibody (ADA) response. All affected animals were female. Concentration data surmised to be ADA-affected were excluded from mean concentration calculations and PK analysis. No meaningful differences in PK parameters were observed between the combination and individually dosed mAbs. A summary of the PK parameters is provided in Table 39. Administration of REGN3479, REGN3471, and REGN3470 individually or in combination as a single 30-minute IV infusion was well tolerated and did not result in any test article-related effects in cynomolgus macaques at individual dose levels of up to 17 mg/kg/day and in combination at a total dose level of up to 50 mg/kg (approximately 16.7 mg/kg/individual REGN test article).

Table 39. Mean Pharmacokinetic Parameters of REGN3479, REGN3471, and REGN3470 Individually or in Combination in the Cynomolgus Macaque

Parameter	Units	REGN3479 17 mg/kg			REGN3471 16.2 mg/kg			REGN3470 16.7 mg/kg			Combination[a] 5 mg/kg						Combination[a] 50 mg/kg					
											Male			Switched Female[b]			Male			Switched Female[c]		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
C_{max}	µg/mL	6	483	74.0	6	545	38.5	6	444	47.2	3	141	14.6	3	145	11.2	3	1360	155	3	1460	265
C_{max}/Dose	(µg/mL)/(mg/kg)	6	28.4	4.35	6	33.6	2.37	6	26.6	2.83	3	28.3	2.91	3	28.9	2.23	3	27.3	3.10	3	29.3	5.30
t_{max}	h	6	0.583	0	6	0.583	0	6	0.583	0	3	0.583	0	3	3	2	3	0.583	0	3	0.583	0
AUC_{inf}	day•(µg/mL)	6	3270	392	4	4660	1200	6	4970	1180	3	963	180	2	796	NC	3	12400	2770	2	14300	NC
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	6	192	23.1	4	288	73.9	6	298	70.8	3	193	35.9	2	159	NC	3	247	55.5	2	287	NC
$t_{1/2}$	day	6	11.4	3.41	4	11.6	2.93	6	16.3	2.35	3	11.9	2.46	2	8.30	NC	3	14.0	2.77	2	16.6	NC
CL	mL/day/kg	6	5.26	0.599	4	3.63	0.821	6	3.50	0.747	3	5.30	0.899	2	6.71	NC	3	4.20	1.06	2	3.61	NC
V_{ss}	mL/kg	6	75.6	17.4	4	53.5	3.36	6	73.6	5.63	3	77.6	8.50	2	64.3	NC	3	72.7	4.92	2	78.3	NC

N = Number of animals; SD = Standard deviation; C_{max} = Peak concentration; t_{max} = Time to C_{max}; AUC_{inf} = Area under the concentration-time curve from time zero extrapolated to infinity; $t_{1/2}$ = Half-life; CL = Total body clearance; V_{ss} = Volume of distribution at steady state; NC = Not calculated
Note: Concentrations likely impacted by ADA were excluded from PK parameter calculations (Table 15).
[a] Combination denotes the total dose of REGN3479, REGN3471, and REGN3470 combined in a 1:1:1 ratio and administered as a single IV infusion.
[b] Switched females from the 50 mg/kg combination group (Section 2.2.2).
[c] Switched females from the 5 mg/kg combination group (Section 2.2.2).

Source: eCtd loc. 4.2.2.2
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Additional Non-GLP ADA Analysis

Late in drug development and after completion of the nonhuman primate (NHP) study, the Applicant developed methods to detect ADA. Stored samples from this study (REGN3479-PK-15086) were tested in three ADA assays to confirm the performance of the developed assays. Test samples from days 1, 21, and 85 (50 mg/kg treated animals only; ~16.7 mg/kg/antibody) were analyzed. Positive responses were detected in postdose samples in two animals (one male and one female), both of which displayed increased clearance consistent with the presence of ADA. One predose sample was detected as positive in the assay. Although it was noted that four animals in this study were considered affected by ADA, only the two REGN-EB3 at 50 mg/kg samples were assessed.

PK in Uninfected Rhesus Macaque

A Single-Dose PK Study of REGN3479, REGN3471 and REGN3470 Following IV Administration to Rhesus Monkeys (Study #R3479-PM-18034)

Table 40. PK in Uninfected Rhesus Macaque Study Design

Methods	Details
Testing facility:	(b) (4)
Dose and frequency of dosing:	See experimental design below; single 1-to-2-minute bolus
Route of administration:	Intravenous
Formulation/vehicle:	Vehicle: 10mM histidine, 10% sucrose, 0.1% PS-80; pH 6.0
Species/strain:	Rhesus macaque
Number/sex/group:	See experimental design below
Age:	2 to 5 years
Satellite groups/unique design:	See experimental design below
Deviations affecting interpretation:	None

Abbreviations: PK, pharmacokinetics; (b) (4)

Table 41. Experimental Design of PK in Uninfected Rhesus Macaque

Group	Number of Animals	Test Article	Dose Route	Target Dose Level (mg/kg)	Target Dose Concentration (mg/mL)	Target Dose Volume (mL/kg)
1	3F	REGN3479	IV	10	10	1
2	3F	REGN3471	IV	10	10	1
3	3F	REGN3470	IV	10	10	1
4	3M/3F	REGN3479/	IV	10	10	1
		REGN3471/		10	10	1
		REGN3470		10	10	1
5	3M/3F	REGN3479/	IV	33.3	50	0.67
		REGN3471/		33.3	50	0.67
		REGN3470		33.3	50	0.67
6	3M/3F	REGN3479/	IV	50	50	1
		REGN3471/		50	50	1
		REGN3470		50	50	1
7	3M/3F	REGN3479/	IV	100	50	2
		REGN3471/		100	50	2
		REGN3470		100	50	2

F Female.
IV Intravenous; given as a bolus injection over approximately 1 to 2 minutes/test article. Groups 4 through 7 were administered each test article sequentially, one test article after another.
M Male.

Source: eCtd loc. 4.2.2.2
Abbreviations: PK, pharmacokinetics; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

To determine the PK of REGN3470, REGN3471, and REGN3479 when administered sequentially to uninfected rhesus macaques as a single IV bolus administration, 21 male and female NHPs received a total dose of 30, 100, 150, or 300 mg/kg (1:1:1). Blood samples for determination of drug concentrations were collected from each animal predose and at 0.083, 4, 8, 24, 48, 72, 120, 168, 240, 336, 432, 504, 672, 840, 1008, 1176, 1344, 1512, 1680, 1848, and 2016 hours postdose.

A precipitous decline in the REGN3470, REGN3471, or REGN3479 concentration was observed in 7 of the 33 animals, consistent with an ADA response. Although no ADA bioanalytical analysis was performed during the study, concentration values that were considered to be impacted by ADA were excluded from mean concentration calculations and data analysis.

Overall, no meaningful differences in PK parameters (Table 42, Table 43, Table 44, Table 45), including maximum plasma concentration (C_{max})/Dose, AUC from time zero extrapolated to infinity (AUC_{inf})/Dose, clearance, and volume of distribution, were observed among REGN3470, REGN3471, and REGN3479 dosed individually or as REGN-EB3, indicating that there were no PK interactions among the three individual mAbs when dosed in combination.

Table 42. Mean Pharmacokinetic Parameters of REGN3470, REGN3471, and REGN3479 in Serum Following Single Sequential Intravenous Administration of 30 mg/kg REGN-EB3 in Rhesus Macaque

REGN-EB3 30 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3470 10 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	164	12.3	7.49	3	175	12.5	7.14	6	170	12.6	7.44
C_{max}/Dose	(µg/mL)/(mg/kg)	3	16.4	1.23	7.49	3	17.5	1.25	7.14	6	17.0	1.26	7.44
t_{max}	h	3	0.0830	0	0	3	0.0830	0	0	6	0.0830	0	0
AUC_{last}	day•(µg/mL)	2	1880	NC	NC	3	1860	254	13.7	5	1870	189	10.1
AUC_{inf}		2	2000	NC	NC	3	1890	259	13.8	5	1930	194	10.0
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	2	188	NC	NC	3	186	25.4	13.7	5	187	18.9	10.1
AUC_{inf}/Dose		2	200	NC	NC	3	189	25.9	13.8	5	193	19.4	10.0
$AUC_{inf\%Extrapolated}$	%	2	5.74	NC	NC	3	1.26	0.344	27.4	5	3.05	4.02	132
$t_{1/2}$	day	2	14.1	NC	NC	3	9.89	4.15	42.0	5	11.6	3.75	32.4
CL	mL/day/kg	2	5.01	NC	NC	3	5.38	0.782	14.5	5	5.23	0.589	11.3
V_{ss}	mL/kg	2	97.4	NC	NC	3	90.8	1.61	1.77	5	93.4	4.59	4.91
REGN-EB3 30 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3471 10 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	149	7.00	4.70	3	166	18.3	11.0	6	158	15.5	9.85
C_{max}/Dose	(µg/mL)/(mg/kg)	3	14.9	0.700	4.70	3	16.6	1.83	11.0	6	15.8	1.55	9.85
t_{max}	h	3	0.0830	0	0	3	1.39	2.26	163	6	0.736	1.60	217
AUC_{last}	day•(µg/mL)	2	1480	NC	NC	3	1530	284	18.5	5	1510	203	13.5
AUC_{inf}		2	1530	NC	NC	3	1550	275	17.7	5	1540	201	13.0
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	2	148	NC	NC	3	153	28.4	18.5	5	151	20.3	13.5
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg	2	153	NC	NC	3	155	27.5	17.7	5	154	20.1	13.0
$AUC_{inf\%Extrapolated}$	%	2	3.38	NC	NC	3	1.37	0.958	70.1	5	2.17	2.48	114
$t_{1/2}$	day	2	11.5	NC	NC	3	9.91	1.91	19.3	5	10.6	1.74	16.4
CL	mL/day/kg	2	6.54	NC	NC	3	6.60	1.30	19.6	5	6.57	0.944	14.4
V_{ss}	mL/kg	2	102	NC	NC	3	87.9	4.15	4.72	5	93.6	8.32	8.89
REGN-EB3 30 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3479 10 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	153	3.06	1.99	3	173	27.6	16.0	6	163	20.5	12.6
C_{max}/Dose	(µg/mL)/(mg/kg)	3	15.3	0.306	1.99	3	17.3	2.76	16.0	6	16.3	2.05	12.6
t_{max}	h	3	0.0830	0	0	3	1.39	2.26	163	6	0.736	1.60	217
AUC_{last}	day•(µg/mL)	2	1350	NC	NC	3	1320	237	17.9	5	1330	187	14.0
AUC_{inf}		2	1390	NC	NC	3	1340	235	17.5	5	1360	180	13.3
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	2	135	NC	NC	3	132	23.7	17.9	5	133	18.7	14.0
AUC_{inf}/Dose		2	139	NC	NC	3	134	23.5	17.5	5	136	18.0	13.3
$AUC_{inf\%Extrapolated}$	%	2	2.40	NC	NC	3	1.44	0.897	62.2	5	1.82	1.58	86.4
$t_{1/2}$	day	2	11.1	NC	NC	3	9.46	1.65	17.4	5	10.1	1.46	14.5
CL	mL/day/kg	2	7.25	NC	NC	3	7.63	1.43	18.7	5	7.48	1.09	14.5
V_{ss}	mL/kg	2	106	NC	NC	3	93.5	7.70	8.23	5	98.5	9.10	9.24

* Concentration values considered to be ADA-impacted were excluded from data analysis (Table 12).
ADA, Anti-drug antibody; AUC, Area under the concentration-time curve; AUC_{inf}, AUC from time zero extrapolated to infinity; $AUC_{inf\%Extrapolated}$, Percentage of AUC extrapolated in the terminal phase to AUC_{inf}, AUC_{last}, AUC computed from time of dosing to the time of last measurable concentration; C_{max}, Peak concentration; CL, Total body clearance; CV, Coefficient of variation; h, Hours; IV, Intravenous; N, Number of animals; NC, Not calculated; SD, Standard deviation; t_{max}, Time to C_{max}; $t_{1/2}$, Elimination half-life; V_{ss}, Volume of distribution at steady state
Note: REGN3470-3471-3479 is also referred to as REGN-EB3 in this document.
Source: eCtd loc. 4.2.2.2
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Table 43. Mean Pharmacokinetic Parameters of REGN3470, REGN3471, and REGN3479 in Serum Following Single Sequential Intravenous Administration of 100 mg/kg REGN-EB3 in Rhesus Macaque

REGN-EB3 100 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3470 33.3 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	783	176	22.5	3	758	12.5	1.65	6	770	112	14.6
C_{max}/Dose	(µg/mL)/(mg/kg)	3	23.5	5.28	22.5	3	22.8	0.375	1.65	6	23.1	3.38	14.6
t_{max}	h	3	0.0830	0	0	3	0.0830	0	0	6	0.0830	0	0
AUC_{last}	day•(µg/mL)	3	7910	693	8.77	3	7350	1090	14.8	6	7630	872	11.4
AUC_{inf}		3	8060	677	8.39	3	7470	1180	15.8	6	7760	918	11.8
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	3	238	20.8	8.77	3	221	32.7	14.8	6	229	26.2	11.4
AUC_{inf}/Dose		3	242	20.3	8.39	3	224	35.4	15.8	6	233	27.6	11.8
$AUC_{inf\%Extrapolated}$	%	3	1.90	0.383	20.2	3	1.53	0.899	58.8	6	1.71	0.650	37.9
$t_{1/2}$	day	3	14.6	0.707	4.86	3	13.8	1.75	12.7	6	14.2	1.27	8.96
CL	mL/day/kg	3	4.15	0.362	8.72	3	4.53	0.654	14.5	6	4.34	0.516	11.9
V_{ss}	mL/kg	3	83.1	11.9	14.3	3	84.0	1.28	1.52	6	83.5	7.60	9.09
REGN-EB3 100 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3471 33.3 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	788	164	20.8	3	730	25.5	3.49	6	759	110	14.5
C_{max}/Dose	(µg/mL)/(mg/kg)	3	23.7	4.93	20.8	3	21.9	0.765	3.49	6	22.8	3.30	14.5
t_{max}	h	3	0.0830	0	0	3	0.0830	0	0	6	0.0830	0	0
AUC_{last}	day•(µg/mL)	3	6640	403	6.07	3	5980	1100	18.5	6	6310	827	13.1
AUC_{inf}		3	6700	399	5.96	3	6010	1130	18.8	6	6360	846	13.3
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	3	199	12.1	6.07	3	180	33.2	18.5	6	189	24.8	13.1
AUC_{inf}/Dose		3	201	12.0	5.96	3	181	33.9	18.8	6	191	25.4	13.3
$AUC_{inf\%Extrapolated}$	%	3	0.886	0.287	32.4	3	0.572	0.328	57.3	6	0.729	0.325	44.6
$t_{1/2}$	day	3	12.2	0.815	6.70	3	10.7	1.66	15.5	6	11.4	1.42	12.5
CL	mL/day/kg	3	4.98	0.292	5.86	3	5.67	1.07	18.9	6	5.33	0.796	14.9
V_{ss}	mL/kg	3	80.0	8.11	10.1	3	79.0	2.03	2.57	6	79.5	5.31	6.68
REGN-EB3 100 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3479 33.3 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	787	158	20.1	3	770	67.9	8.82	6	779	109	14.0
C_{max}/Dose	(µg/mL)/(mg/kg)	3	23.6	4.75	20.1	3	23.1	2.04	8.82	6	23.4	3.28	14.0
t_{max}	h	3	0.0830	0	0	3	0.0830	0	0	6	0.0830	0	0
AUC_{last}	day•(µg/mL)	3	5040	311	6.16	3	5060	1460	28.9	6	5050	946	18.7
AUC_{inf}		3	5070	307	6.04	3	5090	1500	29.4	6	5080	966	19.0
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	3	151	9.33	6.16	3	152	43.9	28.9	6	152	28.4	18.7
AUC_{inf}/Dose		3	152	9.21	6.04	3	153	44.9	29.4	6	153	29.0	19.0
$AUC_{inf\%Extrapolated}$	%	3	0.639	0.139	21.8	3	0.565	0.426	75.5	6	0.602	0.287	47.6
$t_{1/2}$	day	3	12.2	1.37	11.3	3	11.0	2.16	19.7	6	11.6	1.75	15.1
CL	mL/day/kg	3	6.58	0.410	6.23	3	6.90	1.87	27.0	6	6.74	1.22	18.1
V_{ss}	mL/kg	3	91.9	6.95	7.56	3	86.2	4.90	5.68	6	89.1	6.22	6.99

* Concentration values considered to be ADA-impacted were excluded from data analysis (Table 13).
ADA, Anti-drug antibody; AUC, Area under the concentration-time curve; AUC_{inf}, AUC from time zero extrapolated to infinity; $AUC_{inf\%Extrapolated}$, Percentage of AUC extrapolated in the terminal phase to AUC_{inf}; AUC_{last}, AUC computed from time of dosing to the time of last measurable concentration; C_{max}, Peak concentration; CL, Total body clearance; CV, Coefficient of variation; h, Hours; IV, Intravenous; N, Number of animals; SD, Standard deviation; t_{max}, Time to C_{max}; $t_{1/2}$, Elimination half-life; V_{ss}, Volume of distribution at steady state
Note: REGN3470-3471-3479 is also referred to as REGN-EB3 in this document.

Source: eCtd loc. 4.2.2.2
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Table 44. Mean Pharmacokinetic Parameters of REGN3470, REGN3471, and REGN3479 in Serum Following Single Sequential Intravenous Administration of 150 mg/kg REGN-EB3 in Rhesus Macaque

REGN-EB3 150 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3470 50 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	1190	20.8	1.74	3	1020	55.6	5.47	6	1100	104	9.41
C_{max}/Dose	(µg/mL)/(mg/kg)	3	23.9	0.416	1.74	3	20.3	1.11	5.47	6	22.1	2.08	9.41
t_{max}	h	3	0.0830	0	0	3	0.0830	0	0	6	0.0830	0	0
AUC_{last}	day•(µg/mL)	2	11300	NC	NC	3	9320	3360	36.0	5	10100	2610	25.9
AUC_{inf}		2	11400	NC	NC	3	9970	2760	27.7	5	10600	2120	20.1
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	2	225	NC	NC	3	186	67.2	36.0	5	202	52.3	25.9
AUC_{inf}/Dose		2	229	NC	NC	3	199	55.2	27.7	5	211	42.5	20.1
$AUC_{inf\%Extrapolated}$	%	2	1.38	NC	NC	3	8.32	10.1	121	5	5.55	8.08	146
$t_{1/2}$	day	2	13.3	NC	NC	3	13.7	3.88	28.3	5	13.5	2.91	21.5
CL	mL/day/kg	2	4.38	NC	NC	3	5.32	1.66	31.1	5	4.94	1.28	26.0
V_{ss}	mL/kg	2	78.1	NC	NC	3	93.5	8.35	8.93	5	87.3	11.2	12.8
REGN-EB3 150 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3471 50 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	1180	11.5	0.976	2	1130	NC	NC	5	1160	70.5	6.07
C_{max}/Dose	(µg/mL)/(mg/kg)	3	23.7	0.231	0.976	2	22.6	NC	NC	5	23.2	1.41	6.07
t_{max}	h	3	1.39	2.26	163	2	0.0830	NC	NC	5	0.866	1.75	202
AUC_{last}	day•(µg/mL)	2	9640	NC	NC	2	9850	NC	NC	4	9750	479	4.91
AUC_{inf}		2	9710	NC	NC	2	9970	NC	NC	4	9840	472	4.79
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	2	193	NC	NC	2	197	NC	NC	4	195	9.57	4.91
AUC_{inf}/Dose		2	194	NC	NC	2	199	NC	NC	4	197	9.43	4.79
$AUC_{inf\%Extrapolated}$	%	2	0.706	NC	NC	2	1.17	NC	NC	4	0.936	0.389	41.6
$t_{1/2}$	day	2	11.8	NC	NC	2	13.3	NC	NC	4	12.5	1.06	8.46
CL	mL/day/kg	2	5.15	NC	NC	2	5.03	NC	NC	4	5.09	0.236	4.64
V_{ss}	mL/kg	2	76.2	NC	NC	2	86.9	NC	NC	4	81.5	11.3	13.9
REGN-EB3 150 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3479 50 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	1150	32.1	2.79	3	1140	291	25.4	6	1150	185	16.1
C_{max}/Dose	(µg/mL)/(mg/kg)	3	23.1	0.643	2.79	3	22.9	5.82	25.4	6	23.0	3.71	16.1
t_{max}	h	3	0.0830	0	0	3	0.0830	0	0	6	0.0830	0	0
AUC_{last}	day•(µg/mL)	2	6820	NC	NC	3	6470	493	7.62	5	6610	398	6.03
AUC_{inf}		2	6860	NC	NC	3	6590	435	6.60	5	6700	340	5.08
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	2	136	NC	NC	3	129	9.87	7.62	5	132	7.97	6.03
AUC_{inf}/Dose		2	137	NC	NC	3	132	8.70	6.60	5	134	6.80	5.08
$AUC_{inf\%Extrapolated}$	%	2	0.514	NC	NC	3	1.88	1.77	94.3	5	1.33	1.46	110
$t_{1/2}$	day	2	11.4	NC	NC	3	10.5	3.62	34.4	5	10.9	2.65	24.4
CL	mL/day/kg	2	7.29	NC	NC	3	7.61	0.483	6.35	5	7.48	0.383	5.12
V_{ss}	mL/kg	2	92.1	NC	NC	3	99.9	31.4	31.5	5	96.7	23.3	24.0

* Concentration values considered to be ADA-impacted were excluded from data analysis (Table 14).
ADA, Anti-drug antibody; AUC, Area under the concentration-time curve; AUC_{inf}, AUC from time zero extrapolated to infinity; $AUC_{inf\%Extrapolated}$, Percentage of AUC extrapolated in the terminal phase to AUC_{inf}; AUC_{last}, AUC computed from time of dosing to the time of last measurable concentration; C_{max}, Peak concentration; CL Total body clearance; CV, Coefficient of variation; h, Hours; IV, Intravenous; N, Number of animals; NC, Not calculated; SD, Standard deviation; t_{max}, Time to C_{max}; $t_{1/2}$, Elimination half-life; V_{ss}, Volume of distribution at steady state
Note: REGN3470-3471-3479 is also referred to as REGN-EB3 in this document.

Source: eCtd loc. 4.2.2.2
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Table 45. Mean Pharmacokinetic Parameters of REGN3470, REGN3471, and REGN3479 in Serum Following Single Sequential Intravenous Administration of 300 mg/kg REGN-EB3 in Rhesus Macaque

REGN-EB3 300 mg/kg IV (Total Dose)													
Parameter	Unit	REGN3470 100 mg/kg											
		Male				Female				All			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	3	2010	154	7.64	3	1900	77.7	4.10	6	1960	126	6.46
C_{max}/Dose	(µg/mL)/(mg/kg)	3	20.1	1.54	7.64	3	19.0	0.777	4.10	6	19.6	1.26	6.46
t_{max}	h	3	2.69	2.26	83.9	3	0.0830	0	0	6	1.39	2.02	146
AUC_{last}	day•(µg/mL)	3	22900	2970	13.0	3	19100	1710	8.96	6	21000	3000	14.3
AUC_{inf}	day•(µg/mL)	3	23300	3030	13.0	3	19400	1870	9.61	6	21300	3080	14.4
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	3	229	29.7	13.0	3	191	17.1	8.96	6	210	30.0	14.3
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	3	233	30.3	13.0	3	194	18.7	9.61	6	213	30.8	14.4
$AUC_{inf\%Extrapolated}$	%	3	1.72	0.802	46.7	3	1.78	0.700	39.5	6	1.75	0.674	38.6
$t_{1/2}$	day	3	14.1	2.29	16.3	3	14.3	1.30	9.11	6	14.2	1.67	11.8
CL	mL/day/kg	3	4.35	0.580	13.3	3	5.18	0.516	9.95	6	4.77	0.670	14.1
V_{ss}	mL/kg	3	91.5	6.92	7.56	3	102	2.63	2.58	6	96.8	7.46	7.70
REGN-EB3 300 mg/kg IV (Total Dose)													
Parameter	**Unit**	**REGN3471 100 mg/kg**											
		Male				**Female**				**All**			
		N	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**
C_{max}	µg/mL	3	1990	206	10.4	3	1890	75.5	3.99	6	1940	150	7.73
C_{max}/Dose	(µg/mL)/(mg/kg)	3	19.9	2.06	10.4	3	18.9	0.755	3.99	6	19.4	1.50	7.73
t_{max}	h	3	1.39	2.26	163	3	0.0830	0	0	6	0.736	1.60	217
AUC_{last}	day•(µg/mL)	3	18700	2170	11.6	3	13600	1380	10.1	6	16200	3230	20.0
AUC_{inf}	day•(µg/mL)	3	18900	2290	12.1	3	13700	1410	10.3	6	16300	3330	20.4
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	3	187	21.7	11.6	3	136	13.8	10.1	6	162	32.3	20.0
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	3	189	22.9	12.1	3	137	14.1	10.3	6	163	33.3	20.4
$AUC_{inf\%Extrapolated}$	%	3	1.01	0.654	65.0	3	0.525	0.191	36.3	6	0.765	0.505	66.0
$t_{1/2}$	day	3	12.5	2.22	17.7	3	11.2	0.934	8.35	6	11.9	1.69	14.3
CL	mL/day/kg	3	5.34	0.689	12.9	3	7.36	0.718	9.76	6	6.35	1.27	20.0
V_{ss}	mL/kg	3	94.6	0.703	0.744	3	105	6.67	6.34	6	99.9	7.20	7.20
REGN-EB3 300 mg/kg IV (Total Dose)													
Parameter	**Unit**	**REGN3479 100 mg/kg**											
		Male				**Female**				**All**			
		N	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**
C_{max}	µg/mL	3	1960	252	12.9	3	1940	92.9	4.80	6	1950	171	8.76
C_{max}/Dose	(µg/mL)/(mg/kg)	3	19.6	2.52	12.9	3	19.4	0.929	4.80	6	19.5	1.71	8.76
t_{max}	h	3	2.69	2.26	83.9	3	0.0830	0	0	6	1.39	2.02	146
AUC_{last}	day•(µg/mL)	3	13400	1260	9.40	3	8710	646	7.41	6	11100	2730	24.7
AUC_{inf}	day•(µg/mL)	3	13500	1360	10.0	3	8740	651	7.44	6	11100	2800	25.1
AUC_{last}/Dose	day•(µg/mL)/(mg/kg)	3	134	12.6	9.40	3	87.1	6.46	7.41	6	111	27.3	24.7
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	3	135	13.6	10.0	3	87.4	6.51	7.44	6	111	28.0	25.1
$AUC_{inf\%Extrapolated}$	%	3	0.894	0.603	67.4	3	0.378	0.134	35.4	6	0.636	0.482	75.8
$t_{1/2}$	day	3	12.3	2.59	21.1	3	11.0	0.804	7.31	6	11.7	1.86	15.9
CL	mL/day/kg	3	7.43	0.716	9.63	3	11.5	0.824	7.18	6	9.45	2.32	24.6
V_{ss}	mL/kg	3	121	8.73	7.24	3	134	12.0	8.93	6	127	12.0	9.43

AUC, Area under the concentration-time curve; AUC_{inf}, AUC from time zero extrapolated to infinity; $AUC_{inf\%Extrapolated}$, Percentage of AUC extrapolated in the terminal phase to AUC_{inf}; AUC_{last}, AUC computed from time of dosing to the time of last measurable concentration; C_{max}, Peak concentration; CL Total body clearance; CV, Coefficient of variation; h, Hours; IV, Intravenous; N, Number of animals; SD, Standard deviation; t_{max}, Time to C_{max}; $t_{1/2}$, Elimination half-life; V_{ss}, Volume of distribution at steady state
Note: REGN3470-3471-3479 is also referred to as REGN-EB3 in this document.

Source: eCtd loc. 4.2.2.2
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

PK in EBOV-Infected Rhesus Macaque

A Pharmacokinetic/Pharmacodynamic Assessment of REGN3470-3471-3479 in Rhesus Macaque Monkeys Infected with Ebola Virus (Study #R3479-PM-18140)

To determine the PK of REGN3470, REGN3471, and REGN3479, when administered as REGN-EB3 to EBOV-infected rhesus macaques, a blinded and randomized study involving 23 NHPs was performed (Table 46). Animals were challenged with a target dose of 1,000 plaque-forming units (PFUs) intramuscularly (IM) of EBOV Kikwit variant on Day 0 and on Day 5 postexposure, and received a single IV administration of control article or 30, 100, 150, or 300 mg/kg REGN-EB3. The study revealed a difference in survival at only 30 mg/kg (p=0.0072 vs. placebo) and 300 mg/kg (p=0.0025 vs. placebo). Due to the lack of a dose-response relationship, it is unclear how the doses used in this study relate to efficacy, and the data support a claim that survival is likely to be enhanced by REGN-EB3 at doses >30 mg/kg.

Table 46. PK in EBOV-Infected Rhesus Macaque Study Design

Methods	Details
Testing facility:	(b) (4)
Dose and frequency of dosing:	See experimental design below; single 90-second bolus on Day 5
Route of administration:	Intravenous
Formulation/vehicle:	Vehicle: 10mM histidine, 10% sucrose, 0.1% PS-80; pH 6.0
Species/strain:	Rhesus macaque
Number/sex/group:	See experimental design below
Age:	3.6 to 5.6 years
Satellite groups/unique design:	See experimental design belowabove
Deviations affecting interpretation:	Yes; Significant number of study deviations without an adequate inspection of study records to determine impact.
Animal prescreen:	Appropriate
Challenge strain:	Ebola virus (Kikwit variant), passage number 2 on Vero E6 cells (Ebola virus *H.sapiens*-tc/COD/1995/Kikwit-9510621, species *Zaire ebolavirus*) acquired from (b) (4)
Challenge procedures:	Five days after acclimation at ABSL-4 IM injection of EBOV Kikwit in a 0.5 mL volume Planned exposure dose of 1,000 PFU
Euthanasia criteria:	This study evaluated survival. Nonsurvival was defined as terminal illness or being moribund. Animal health was evaluated daily using a clinical observation scoring system (below). Animals were to be humanely euthanized if any of the following conditions are met: 1) Total clinical score ≥15 2) Prostrate but able to rise if stimulated, moderate to dramatically reduced response to external stimuli with ≥5°F temperature change from baseline 3) Prostrate but able to rise if stimulated, moderate to dramatically reduced response to external stimuli, and if any two of the following are observed (from most recent blood draw): ALT >200 U/L, ALP >1100 U/L, GGT >170 U/L, BUN >30 mg/dL, ALB <3.0 g/dL

Abbreviations: ALB, a bumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; EBOV, *Zaire ebolavirus;* GGT, gamma-glutamyl transpeptidase; IM, intramuscularly; PFU, plaque-forming unit; PK, pharmacokinetics; (b) (4)

Table 47. PK in EBOV-Infected Rhesus Macaque—Experimental Design

Groups		Virus Exposure		Treatment Dosing		
Number	*Size*	*EBOV Kikwit Dose (IM)*	*Schedule (Day[1])*	*Therapeutic Agent*	*Dose (IV)*	*Schedule (Day[1])*
1	4	1,000 PFU	0	Placebo REGN3470-3471-3479	0 mg/kg	5
2	5	1,000 PFU	0	REGN3470-3471-3479 cocktail	30 mg/kg (10 mg/kg per mAb)	5
3	5	1,000 PFU	0	REGN3470-3471-3479 cocktail	100 mg/kg (33.3 mg/kg per mAb)	5
4	4[2]	1,000 PFU	0	REGN3470-3471-3479 cocktail	150 mg/kg (50 mg/kg per mAb)	5
5	5	1,000 PFU	0	REGN3470-3471-3479 cocktail	300 mg/kg (100 mg/kg per mAb)	5

[1] relative to the Ebola virus exposure; [2] Animal removed due to health complication was originally randomized into group 4, see Study Deviation Report No. 01; IV = intravenous; IM = intramuscular; PFU = plaque forming units; mAb = monoclonal antibody.
Source: eCtd loc. 4.2.2.2
Abbreviations: EBOV, *Zaire ebolavirus;* PK, pharmacokinetics

Table 48. Clinical Observation Scoring System in EBOV-Infected Rhesus Macaque

Weight loss[1,5]. 0: 0% to <10%↓. 1: ≥10% to <20%↓. 2: ≥ 20%↓.	0 1 2 - CO
Temperature Change[1,5]. 0: < 2°F. 1: ≥ 2°F. 2:≥ 3°F. 3: ≥ 5°F.	0 1 2 3 - CO
RESPONSIVENESS 0 = Alert, responsive, normal activity, free of disease signs or exhibits only resolved/resolving disease signs **1** = Slightly diminished general activity, subdued but responds normally to external stimuli; **2** = Withdrawn, may have head down, fetal posture, hunched, reduced response to external stimuli; **8** = Prostrate but able to rise if stimulated, moderate to dramatically reduced response to external stimuli; **15** = Persistently prostrate, severely or completely unresponsive, may have signs of respiratory distress	0 1 2 8 15
Hair coat. 0: = normal appearance. 1: = rough hair coat	0 1
Respiration. 0: normal breathing. 8: labored. 15: Agonal.	0 8 15
Petechia[4] 0: none. 1: mild,1-39%. 2: moderate, 40-79%. 3: severe,≥80%	0 1 2 3 CO
Bleeding. 0: none. 1: at bleeding site. 2: other then bleeding site	0 1 2
Nasal discharge. 0: not present. 1: present	0 1
Feed eaten[6]. 0: 100% -25% 1: <25%	0 1 CO
Food enrichment[6]. 0: 100% - 25% 1: <25%	0 1 CO
Stool: 0: normal. 1: no stool present. 2D: diarrhea 2R: decreased	0 1 2D 2R
Fluid Intake. 0: drinking. 1: reduced fluid intake. 2: not drinking.	0 1 2
Dehydration[1]. 0: Normal, 1:≥2sec	0 1 CO
Total Clinical Score[2] =	
Data Review (to be done at each observation time) Initial / Date:	

[1] Only performed during sedation
[2] Total Clinical Score is determined by adding up all Clinical scores. Clinical scores are reported to responsible veterinarian daily. If clinical score is ≥5, the animal is reported to study veterinarian for evaluation. If total score is ≥15, animal is considered "terminally ill" and should be euthanized. Exceptions require consultation and approval by study veterinarian. Additional Euthanasia criteria: Humanely euthanize the animals as soon as any of the following: 1) Prostrate but able to rise if stimulated, moderate to dramatically reduced response to external stimuli with equal or greater than 5 degree change from baseline or 2) Prostrate but able to rise if stimulated, moderate to dramatically reduced response to external stimuli and if any two of the following are true: ALT >200; ALP>1100; GGT>170; BUN>30; ALB<3.0. the veterinarian will approve all euthanasia
[3] Day relative to challenge
[4] Please document any Petechia score above 0 on Petechia Chart;
[5] Data must be retaken on these animals prior to euthanasia to confirm score
[6] See Daily Feed Consumption Report
Source: eCtd loc. 4.2.2.2
Abbreviation: EBOV, *Zaire ebolavirus;*

Table 49. EBOV-Infected Rhesus Macaque Study Review Findings

Parameter	Major Findings
Animal Model:	Not well characterized; appropriate natural history information from this facility unavailable.
Euthanasia Criteria:	Not scientifically justified.
Clinical Scoring System:	Facility SOP. Not justified via characterization of Ebola disease symptoms in the model.
Consensus on Study Design/Conduct:	DAV reviewed draft study protocol and provided recommendations to improve study design. No consensus was reached for critical aspects of study design in the final completed protocol.
Statistical Considerations:	Study was not powered to reach definitive conclusions, except for the primary endpoint of survival (prevention of mortality) in treated vs. control animals. Breadth of activity, including the ability to determine optimal dose level, would require significantly more animals then outlined in the final completed protocol.

Abbreviations: EBOV, *Zaire ebolavirus;* SOP, standard operating procedure

The PK analysis determined the exposure of each component of REGN-EB3. The parameters are presented in Table 50. A precipitous decline in REGN3470, REGN3471, or REGN3479 concentrations in 7 of the 19 (37%) drug-treated animals (3 of 5, 2 of 5, 1 of 4, and 1 of 5 for the 30 mg/kg, 100 mg/kg, 150 mg/kg, and 300 mg/kg REGN-EB3 cocktail groups, respectively) was observed, consistent with an ADA response. Although no ADA bioanalytical analysis was performed, concentration values that were considered to be impacted by ADA were excluded from mean concentration calculations and data analysis. ADA appeared to impact systemic concentrations on Day 26 (21 days posttreatment), suggesting that the presence of ADA did not have a meaningful impact on activity since viral titers typically peaked around Day 7 and were undetectable by Day 21.

Table 50. Mean Pharmacokinetic Parameters of REGN3470, REGN3471, and REGN3479 in Serum Following Single Intravenous Administration of REGN-EB3 in EBOV-Infected Rhesus Macaque

Parameter	Unit	REGN-EB3 30 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	5	198	47.0	23.8	5	188	40.6	21.6	5	166	49.7	30.0
C_{max}/Dose	(µg/mL)/(mg/kg)	5	19.8	4.70	23.8	5	18.8	4.06	21.6	5	16.6	4.97	30.0
t_{max}	h	5	0.0833	0	0	5	0.0833	0	0	5	4.87	10.7	220
AUC_{inf}	day•(µg/mL)	5	868	572	65.9	5	551	420	76.3	5	712	469	65.8
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	5	86.8	57.2	65.9	5	55.1	42.0	76.3	5	71.2	46.9	65.8
$t_{1/2}$	day	5	3.24	2.38	73.3	5	2.14	1.35	63.3	5	2.80	2.12	75.8
CL	mL/day/kg	5	22.5	22.8	101	5	41.5	43.5	105	5	25.9	24.6	95.1
V_{ss}	mL/kg	5	49.4	11.3	22.8	5	56.5	13.1	23.2	5	52.7	17.7	33.6
Parameter	Unit	REGN-EB3 100 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	5	801	131	16.4	5	734	150	20.5	5	794	125	15.7
C_{max}/Dose	(µg/mL)/(mg/kg)	5	24.0	3.93	16.4	5	22.0	4.51	20.5	5	23.9	3.74	15.7
t_{max}	h	5	0.0833	0	0	5	0.0833	0	0	5	0.0833	0	0
AUC_{inf}	day•(µg/mL)	5	3110	1670	53.8	5	1860	1420	76.4	5	2150	942	43.8
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	5	93.3	50.2	53.8	5	56.0	42.8	76.4	5	64.5	28.3	43.8
$t_{1/2}$	day	5	4.17	2.88	69.1	5	2.01	1.89	94.2	5	2.90	2.05	70.8
CL	mL/day/kg	5	16.0	13.8	86.4	5	36.6	33.0	90.2	5	20.1	13.7	68.2
V_{ss}	mL/kg	5	54.0	13.2	24.4	5	43.6	22.0	50.4	5	52.5	20.7	39.4
Parameter	Unit	REGN-EB3 150 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	4	1120	448	40.0	4	1030	410	39.7	4	1070	378	35.3
C_{max}/Dose	(µg/mL)/(mg/kg)	4	22.4	8.96	40.0	4	20.6	8.20	39.7	4	21.4	7.55	35.3
t_{max}	h	4	0.0833	0	0	4	0.0833	0	0	4	0.0833	0	0
AUC_{inf}	day•(µg/mL)	4	4400	2420	55.1	4	3410	2260	66.2	4	3190	1600	50.1
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	4	88.1	48.5	55.1	4	68.2	45.1	66.2	4	63.8	32.0	50.1
$t_{1/2}$	day	4	4.01	3.29	82.1	4	3.53	3.72	105	4	3.37	3.02	89.5
CL	mL/day/kg	4	14.7	8.06	55.0	4	24.4	21.9	89.9	4	19.6	10.8	55.1
V_{ss}	mL/kg	4	57.8	24.6	42.6	4	60.8	31.5	51.9	4	57.1	22.5	39.3

Parameter	Unit	REGN-EB3 300 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
		N	Mean	SD	CV%	N	Mean	SD	CV%	N	Mean	SD	CV%
C_{max}	µg/mL	5	2050	478	23.3	5	2040	452	22.2	5	2030	466	23.0
C_{max}/Dose	(µg/mL)/(mg/kg)	5	20.5	4.78	23.3	5	20.4	4.52	22.2	5	20.3	4.66	23.0
t_{max}	h	5	0.0833	0	0	5	0.0833	0	0	5	0.0833	0	0
AUC_{inf}	day•(µg/mL)	5	18500	3690	20.0	5	15000	2340	15.6	5	10700	1940	18.2
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	5	185	36.9	20.0	5	150	23.4	15.6	5	107	19.4	18.2
$t_{1/2}$	day	5	9.54	3.19	33.5	5	7.93	1.71	21.5	5	7.01	1.36	19.3
CL	mL/day/kg	5	5.64	1.40	24.9	5	6.77	1.03	15.2	5	9.61	1.75	18.2
V_{ss}	mL/kg	5	73.6	17.3	23.5	5	72.8	15.2	20.9	5	82.9	21.4	25.8

* Concentration values considered to be ADA impacted were excluded from data analysis (Table 9 through Table 12)
ADA, Anti-drug antibodies; AUC, Area under the concentration-time cure; AUC_{inf}, AUC from time zero extrapolated to infinity; CL, Total body clearance; C_{max}, Peak concentration; CV, Coefficient of variation; EBOV, Ebola virus; h, Hours; N, Number of animals; SD, Standard deviation; $t_{1/2}$, Elimination half-life; V_{ss}, Volume of distribution at steady state
Note: REGN3470-3471-3479 is also known as REGN-EB3 in this document.

Source: eCtd loc. 4.2.2.2
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

The concentration-time profiles of REGN3470, REGN3471, or REGN3479 following a single IV administration of REGN-EB3 cocktail to the EBOV-infected rhesus macaques are suggestive of target-mediated clearance, with high viral load having an impact on PK, especially at the lower REGN-EB3 dose levels. See the virology review (Section III.18.1) for additional details on viral load and secreted glycoprotein (sGP) assessments. A high viral load and a high sGP at baseline on Day 5 (prior to treatment) were associated with a lower survival rate of treated animals.

13.1.4. Toxicology

13.1.4.1. General Toxicology

Three-Week Intravenous Toxicity Study in Rats (Study #REGN3479-TX-15001)

Key Study Findings

- No observed adverse effect level=195 mg/kg/dose [65 mg/kg/dose for each component]
- AUC_{tau}=84.7 mg.day/mL; C_{max} =11.1 mg/mL
- No adverse, drug-related toxicities were observed up to the highest dose tested.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 51. Three-Week Rat IV Toxicity Study Design

Methods	Details
Dose and frequency of dosing:	See experimental design below; Slow bolus on days 1, 8, and 15
Route of administration:	Intravenous
Formulation/vehicle:	Vehicle: 10mM histidine, 10% sucrose, 0.1% PS-80; pH 6.0 Diluent: Saline
Species/strain:	Sprague-Dawley rats
Number/sex/group:	See experimental design below
Age:	10/11 weeks
Satellite groups/unique design:	See experimental design below
Deviations affecting interpretation:	None

Abbreviations: IV, intravenous

Table 52. Experimental Design of 3-Week Rat IV Toxicity Study

Group No.	Test Material	Dose Level (mg/kg/dose)[a]	Number of Animals (M / F)			
			Terminal[b]	Recovery[c]	TK	Cardiovascular (M only)[d]
1	Control	0	10/10	5/5	3/3	5
2	REGN3479	15	10/10	5/5	9/9	NA
3	REGN3471	15	10/10	5/5	9/9	NA
4	REGN3470	15	10/10	5/5	9/9	NA
5	REGN3479, REGN3471, REGN3470	5 5 5	10/10	5/5	9/9	5
6	REGN3479, REGN3471, REGN3470	15 15 15	10/10	5/5	9/9	NA
7	REGN3479, REGN3471, REGN3470	65 65 65	10/10	5/5	9/9	5

M = male; F = female; TK = toxicokinetic; NA = not applicable.
Note: Control animals were administered the control article over 8-9 minutes. Groups 2-4 were administered each monoclonal antibody individually over 3 minutes. Groups 5-7 were administered sequential doses of all three antibodies (REGN3479, then REGN3471, and then REGN3470) over a 9-minute period (3 minutes each dose) via IV injection.
[a] Total combined doses for the three monoclonal antibodies in Groups 5, 6 and 7 of 15 mg/kg/dose, 45 mg/kg/dose and 195 mg/kg/dose, respectively.
[b] Main study animals assigned for terminal necropsy were euthanized on Day 19.
[c] Recovery animals were euthanized on Day 85.
[d] Cardiovascular animals were only evaluated for telemetry recordings.
Source: eCtd loc. 4.2.3.2
Abbreviation: IV, intravenous

Table 53. Three-Week Rat IV Toxicity Study Findings

Parameter	Major Findings
Mortality	No unscheduled deaths.
Clinical signs	Examined at least once daily. No drug-related findings.
Body weights	Measured weekly. No drug-related findings.
Food consumption	Measured weekly. No drug-related findings.
Ophthalmoscopy	Evaluated pretreatment and within 1 week of necropsy. No drug-related findings.
Hematology	Evaluated pretreatment and days 19 and 85. No drug-related findings.
Clinical chemistry	Evaluated on days 19 and 85. Decreased glucose (0.70-0.79×) in animals dosed ≥15 mg/kg/dose REGN3470-3471-3479. Increased phosphorus (1.19-1.39×) on Day 19 in animals dosed ≥15 mg/kg/dose. Decreased creatine kinase activity (0.21-0.38×) on Day 19 in animals dosed with ≥15 mg/kg/dose REGN3470-3471-3479 and on Day 85 of males dosed with 15 mg/kg/dose of each mAb alone. These findings were considered nonadverse due to low severity and no apparent pathophysiologic mechanism.
Gross pathology	Evaluated at necropsy (Days 19 and 85). No drug-related findings.
Organ weights	Evaluated at necropsy (Days 19 and 85). No drug-related findings.
Histopathology Adequate battery: Yes Peer review: Yes	Evaluated at necropsy. No drug-related findings.
Cardiovascular Assessment	Evaluated pretreatment, on Day 19, and within 1 week of necropsy. No drug-related findings.

Abbreviations: IV, intravenous

Table 54. Toxicokinetic Data

Study Title (Study No.)	Major Findings
General Toxicology Studies	
Three-Week Intravenous Toxicity Study in Rats (Study #REGN3479-TX-15001) Sample collection times: 0.08, 6, 24, 48, 72, 168, and 240 hours and Day 14 NOAEL =195 mg/kg/day ($AUC_{tau+rec}$=72.2 mg.day/mL at Day 3, gender-averaged) **Exposure multiple=1.2** Based on mean steady-state exposures in healthy human volunteers receiving 150 mg/kg REGN-EB3 (AUC_{0-inf}=61.4 mg.day/mL)	See table below

Parameter	Units	Dose	REGN3479 15 mg/kg	REGN3471 15 mg/kg	REGN3470 15 mg/kg	Combination[a] 15 mg/kg (5mg/kg per compound)	Combination[a] 45 mg/kg (15mg/kg per compound)	Combination[a] 195 mg/kg (65mg/kg per compound)
C_{max}	µg/mL	1	480	594	460	539	1550	8150
		3	718	821	683	778	2380	11100
C_{max}/Dose	(µg/mL)/(mg/kg)	1	32.0	39.6	30.7	35.9	34.3	41.8
		3	47.9	54.7	45.5	51.9	52.8	56.8
t_{max}	hour	1	0.0833	0.0833	0.0833	0.0833	0.0833	0.0833
		3	0.0833	0.0833	0.0833	0.0833	0.0833	0.0833
AUC_{tau}[b]	day•(µg/mL)	1	1200	1420	1180	1450	4050	16100
		3	2280	2370	2250	2410	6610	23400
AUC_{Rec}[c]			4780	4950	5050	5020	14200	48800
AUC_{tau}/Dose	day•(µg/mL)/(mg/kg)	1	80.0	94.7	78.7	96.7	90.0	82.6
		3	152	158	150	161	147	120
AUC_{Rec}/Dose			319	330	337	335	316	250
C_{trough}	µg/mL	1	111	152	111	147	405	1300
		2	195	234	200	213	638	1820
		3	232	234	227	188	608	1870
$t_{1/2}$	day	3	9.18	8.31	10.2	9.39	9.10	10.5

C_{max} = Peak concentration; t_{max} = Time to C_{max}; AUC_{tau} = Area under the concentration-time curve calculated during the dosing interval; AUC_{Rec} = Area under the concentration-time curve calculated during the recovery phase; C_{trough} = Trough concentration; $t_{1/2}$ = half-life
[a] "Combination" equals total dose of administered drug and denotes that serial doses of all three antibodies (REGN3479, then REGN3471, then REGN3470) were administered. Dose-normalized parameters (C_{max}/Dose and AUC/Dose) were normalized by the total amount of the REGN3479, REGN3471 and REGN3470 doses administered.
[b] For dose 1, AUC_{tau} denotes study days 1-8. For dose 3, AUC_{tau} denotes study days 15-22.
[c] AUC_{Rec} denotes study days 15-85 over the entire study duration.

Source: eCtd loc. 4.2.3.2

Abbreviations: NOAEL, no observed adverse effect level; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Tissue Cross-Reactivity Study in Adult Tissues (Study #REGN3479-TX-15005)

Key Study Findings

- No clinically relevant reactivity was observed in the study.
- No staining was observed with Biotin-REGN3470 in any human or rat tissues.
- Staining of Biotin-REGN3479 was present in both human and rat tissues.
- Only Biotin-REGN3471 had staining in human tissue that was not present in rat tissues.
- All staining observed (Biotin-REGN3479 and Biotin-REGN3471) was cytoplasmic and not toxicologically meaningful.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 55. TCR in Adult Tissue Study Design

Method	Details
Tissues:	Cryosections of normal human and rat tissues from three donors per tissue
Dosing concentrations:	0.5 and 2.5 µg/mL Biotin-REGN3470, Biotin-REGN3471 2 and 10 µg/mL Biotin-REGN3479 0.5, 2, 2.5, and 10 µg/mL Biotin-HuIgG1
Positive control:	Recombinant Zaire Ebola Glycoprotein UV-resin spot slides (spotted at 20 µg/mL), designated EBOV-G
Negative control:	Human hypercalcemia of malignancy peptide, amino acid residues 1 to 34, UV-resin spot slides (spotted at 20 µg/mL), designated PTHrP 1-34
Control article:	Biotinylated human IgG1 (Biotin-HuIgG1)
Staining procedure and incubation time:	Biotinylated Abs were applied to tissues for 1 hour; washed, treated with ABC Elite for 30 minutes, washed, and treated with DAB for 4 minutes

Method	Details
Tissue suitability:	Staining for von Willebrand Factor
Tissues examined:	See table below

Adrenal	Heart	Salivary Gland
Bladder (urinary)	Kidney (glomerulus, tubule)	Skin
Blood Cells [a]	Liver	Spinal Cord
Blood Vessels (endothelium) [b]	Lung	Spleen
Bone Marrow	Lymph Node	Striated Muscle (skeletal)
Brain – cerebellum	Ovary	Testis
Brain – cerebrum (cerebral cortex)	Pancreas	Thymus
Breast	Parathyroid	Thyroid
Colon (large intestine)	Peripheral Nerve	Tonsil
Eye	Pituitary	Ureter
Fallopian Tube	Placenta	Uterus – cervix
Gastrointestinal (GI) Tract [c]	Prostate	Uterus – endometrium

[a] Evaluated from peripheral blood smears.
[b] Evaluated from all tissues where present.
[c] Includes esophagus, small intestine, and stomach (including underlying smooth muscle).

Source: eCtd loc. 4.2.3.7.7

Abbreviations: Abs, antibodies; DAB, diaminobenzidine; EBOV-G, Ebola glycoprotein; IgG1, immunoglobulin G1; PTHrP, parathyroid hormone-related protein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; TCR, tissue cross-reactivity; UV, ultraviolet

Table 56. TCR Findings in Adult Tissue

Parameter	Major Findings
Suitability	All controls functioned properly, and tissues were suitable for inclusion.
Membrane staining	No relevant findings.
Cytoplasmic staining	Biotin-REGN3470: No relevant findings. Biotin-REGN3471: Stained the ductal epithelium in the breast, esophagus, and pancreas; glandular epithelium in the esophagus (submucosal glands) and uterus (endocervical glands and endometrial glands); mucosal epithelium in the colon and stomach; bronchiolar epithelium in the lung; and acinar epithelium in the salivary gland. • Staining of epithelium (that usually had the appearance of mucus-producing epithelia) varied in intensity and frequency. • Staining was present at 2.5 and 0.5 µg/mL with reduced intensity and/or frequency at the lower concentration. • No staining was observed with Biotin-REGN3471 in any rat tissues examined. Biotin-REGN3479: Stained the ductal epithelium in the breast; corneal epithelium in the eye; mucosal epithelium (basal squamous layer) in the esophagus, tonsil, ureter, and cervix; epithelium of the prostate; epithelium of the skin (sweat glands, sebaceous glands, hair follicles, and epidermis); epithelial-reticular cells in the thymus; and crypt epithelium in the tonsil. • Staining of epithelium was weak or weak-to-moderate in intensity and varied in frequency. • Staining was present only at the higher concentration (10 µg/mL). • Staining was observed more in human tissues than rat tissues.

Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; TCR, tissue cross-reactivity

Tissue Cross-Reactivity Study in Fetal Human Tissues (Study #REGN3479-TX-18122):

Key Study Findings

- No staining was observed with Biotin-REGN3470 in any tissue.
- Staining of Biotin-REGN3479 and Biotin-REGN3471 was present in tissues.
- Membrane reactivity that could be clinically relevant was observed in the cervix, lung, eye, and thymus.

- Regeneron suggests that the membrane staining observed with REGN3471-Bio and REGN3479-Bio and its translatability to in vivo cross-reactivity are not clear.
- Regeneron performed a follow-up study to determine the specificity of the observed staining (see Study #REGN3479-TX-200001).

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 57. TCR in Fetal Tissue Study Design

Method	Details
Tissues:	Cryosections of fetal human tissue from up to three donors per tissue
Dosing concentrations:	0.5 and 2.5 µg/mL Biotin-REGN3470, Biotin-REGN3471 2 and 10 µg/mL Biotin-REGN3479 0.5, 2, 2.5, and 10 µg/mL Biotin-HuIgG1
Positive control:	Recombinant Zaire Ebola Glycoprotein UV-resin spot slides (spotted at 20 µg/mL), designated EBOV-G
Negative control:	Human hypercalcemia of malignancy peptide, amino acid residues 1 to 34, UV-resin spot slides (spotted at 20 µg/mL), designated PTHrP 1-34
Control article:	Biotinylated human IgG1 (Biotin-HuIgG1)
Staining procedure and incubation time:	Biotinylated Abs were applied to tissues for 1 hour; washed, treated with ABC Elite for 30 minutes, washed, and treated with DAB for 4 minutes
Tissue suitability:	Immunohistochemistry of β2-microglobulin

Method	Details
Tissues examined:	

Fetal Human Tissue	Donor Number	Gestation of Donor at Tissue Collection
Adrenal	HT204	22 weeks
Adrenal	HT320	22 weeks
Bone Marrow	HT208	23.5 weeks
Brain	HT207	22 weeks
Brain	HT244	26 weeks
Brain	HT1822	24 weeks
Cervix	HT1452	26 weeks
Cervix	HT1453	25 weeks
Esophagus	HT204	22 weeks
Esophagus	HT206	23 weeks
Esophagus	HT320	22 weeks
Eye	HT204	22 weeks
Eye	HT244	26 weeks
Eye	HT320	22 weeks
Heart	HT204	22 weeks
Heart	HT1452	26 weeks
Heart	HT1455	25 weeks
Kidney	HT204	22 weeks
Kidney	HT320	22 weeks
Large Intestine	HT206	23 weeks
Liver	HT208	23.5 weeks
Liver	HT209	24 weeks
Liver	HT320	22 weeks
Lung	HT206	23 weeks
Lung	HT207	22 weeks
Lung	HT320	22 weeks
Muscle	HT208	23.5 weeks
Muscle	HT209	24 weeks
Muscle	HT320	22 weeks
Pancreas	HT204	22 weeks
Pancreas	HT325	22 weeks
Pancreas	HT1824	20 weeks
Skin	HT206	23 weeks
Skin	HT244	26 weeks
Skin	HT1463	29 weeks
Small Intestine	HT320	22 weeks
Small Intestine	HT897	23 weeks
Spleen	HT204	22 weeks
Spleen	HT206	23 weeks
Spleen	HT320	22 weeks
Stomach	ST-0026A[1]	38 weeks
Thyroid	HT244	26 weeks
Thymus	TY-0032A[1]	38 weeks
[1] Tissue sections provided by (b) (4)		

Source: eCtd loc. 4.2.3.7.7

Abbreviations: Abs, antibodies; DAB, diaminobenzidine; EBOV-G, Ebola glycoprotein; IgG1, immunoglobulin G1; PTHrP, parathyroid hormone-related protein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; TCR, tissue cross-reactivity; UV, ultraviolet

Table 58. TCR Findings in Fetal Tissue

Parameter	Major Findings
Suitability	All controls functioned properly, and tissues were suitable for inclusion.
Membrane staining	Biotin-REGN3470: No relevant findings. Biotin-REGN3471: Stained the epithelium in the fetal cervix (internal ostium) and lung (bronchi and bronchial glands). • Staining of epithelium was weak-to-moderate or moderate-to-strong in intensity and varied in frequency. • Staining was present at 2.5 and 0.5 µg/mL with similar or reduced intensity and/or frequency at the lower concentration. Biotin-REGN3479: Stained the conjunctival epithelium in the fetal eye, bronchial and bronchial glandular epithelium in the fetal lung, and epithelial-reticular cells in the fetal thymus. • Staining of epithelium was weak-to-moderate or weak-to-strong in intensity and varied in frequency. • Staining was present only at the higher concentration (10 µg/mL).

Parameter	Major Findings
Cytoplasmic staining	Biotin-REGN3470: No relevant findings. Biotin-REGN3471: Stained the mucosal epithelium in the fetal large intestine and stomach. Biotin-REGN3479: Stained the epidermal epithelium and root sheaths of hair follicles in the fetal skin.

Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; TCR, tissue cross-reactivity

Competition/Tissue Cross-Reactivity Study with REGN3471 and REGN3479 in Fetal/Adult Human Tissues (Study #REGN3479-TX-20001)

Key Study Findings

- Staining of Biotin-REGN3479 and Biotin-REGN3471 was blocked when REGN3479 and REGN3471, respectively, was added prior to application of the biotinylated Abs.
- Preincubation with REGN3471 and REGN3479 did not prevent membrane reactivity of the biotinylated Abs to fetal tissues observed in the lung, eye, and thymus.
- Suggest observed membrane staining in fetal tissues is the result of nonspecific binding.

Conducting laboratory: (b) (4)

GLP compliance: No

Table 59. Competition/TCR in Adult and Fetal Tissue Study Design

Method	Details
Tissues:	Cryosections of human tissue from up to two donors per tissue for adults and a single donor for fetal tissues.
Dosing concentrations:	2.5 µg/mL Biotin-REGN3471 10 µg/mL Biotin-REGN3479 10 µg/mL Biotin-HuIgG1
Positive control:	Recombinant Zaire Ebola Glycoprotein UV-resin spot slides (spotted at 10 µg/mL), designated EBOV-G
Negative control:	Human hypercalcemia of malignancy peptide, amino acid residues 1 to 34, UV-resin spot slides (spotted at 10 µg/mL), designated PTHrP 1-34
Control Article:	Biotinylated Human IgG1 (Biotin-HuIgG1)
Competition assay:	Following protein block, 250 µg/mL REGN3471; 500 µg/mL REGN3479; 200 µg/mL HuIgG1 for 1 hour. Rinsed prior to biotinylated Abs staining procedure.
Staining procedure and incubation time:	Biotinylated Abs were applied to tissues for 1 hour, washed, treated with ABC Elite for 30 minutes, washed, and treated with DAB for 5 minutes.
Tissue suitability:	Reviewed by pathologist
Adult tissues examined:	Tissues for REGN3471 Breast (mammary gland) / Lung / Stomach Esophagus / Pancreas / Uterus – cervix Large Intestine/Colon / Salivary Gland / Uterus – endometrium Tissues for REGN3479 Adrenal / Lymph Node / Striated Muscle (skeletal) Bladder (urinary) / Peripheral Nerve / Thymus Breast (mammary gland) / Placenta / Tonsil Esophagus / Prostate / Ureter Eye / Skin / Uterus – cervix Heart / Spinal Cord / Uterus – endometrium Liver / Spleen

Method	Details
Fetal tissues examined:	Tissues for REGN3471

Fetal Human Tissue	Donor Number	Gestation of Donor at Tissue Collection
Cervix[1]	HT1452	26 weeks
Large Intestine	HT206	23 weeks
Lung	HT206	23 weeks
Stomach	ST-0026A[2]	38 weeks
[1] Tissue was no longer available in the Testing Facility tissue bank for inclusion in the study. [2] Tissue sections were provided by (b) (4)		

Tissues for REGN3479

Fetal Human Tissue	Donor Number	Gestation of Donor at Tissue Collection
Eye	HT204	22 weeks
Lung	HT206	23 weeks
Skin	HT244	26 weeks
Thymus	TY-0032A[1]	38 weeks
[1] Tissue sections were provided by (b) (4)		

Source: eCtd loc. 4.2.3.7.7

Abbreviations: Abs, antibodies; DAB, diaminobenzidine; EBOV-G, Ebola glycoprotein; IgG1, immunoglobulin G1; PTHrP, parathyroid hormone-related protein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; TCR, tissue cross-reactivity; UV, ultraviolet

Table 60. TCR Findings in Fetal Tissue

Parameter	Major Findings
Suitability	All tissues were not suitable for inclusion. Tissues were replaced per pathologist recommendation, except for the large intestine/colon adult tissue, of which there was only one donor sample.
Competition assay phase	Biotin-REGN3471: • Stained the positive control. • Staining was reduced by prior application of REGN3471, indicating partial/almost complete blocking. Biotin-REGN3479: • Stained the positive control. • Staining was eliminated by prior application of REGN3479, indicating complete blocking. Biotin- HuIgG1 • No staining was observed.
Fetal human tissue binding	Biotin-REGN3471: • Staining of extracellular material in the large intestine; slightly reduced in intensity and frequency with REGN3471 competition. • Staining of the membrane of epithelial cells of the lung; staining was unaffected by REGN3471 competition. • Staining of the mucosal epithelium in the stomach; staining was unaffected by REGN3471 competition. Biotin-REGN3479: • Staining of epithelial cells in the cornea and conjunctiva; staining was unaffected by REGN3479 competition. • Staining of the membrane of epithelial cells of the lung; staining was unaffected by REGN3479 competition. • Staining of the epithelial cells in the skin; staining was unaffected by REGN3479 competition. • Staining of the epithelial-reticular cells in the thymus; staining was unaffected by REGN3479 competition.

Parameter	Major Findings
Adult human tissue binding	Tissues were not examined for Biotinylated Ab alone in this assay. Biotin-REGN3471 with pre- REGN3471: • Staining was observed in cytoplasm of the endocervical glandular epithelium, the mucosal epithelium of large intestine/colon, the ductal epithelium, and the glandular epithelium in the endometrium. • No staining was observed in the adult esophagus, lung, breast (mammary gland), salivary gland, or stomach. Biotin-REGN3479 with pre- REGN34719: • Staining was observed in cytoplasm of bladder, cervix, esophagus, eye, breast (mammary gland), prostate, skin, thymus, tonsil, and ureter cells. Cytoplasm of spindloid/dendritic/macrophage cells also stained in multiple tissues. • No staining was observed in the adrenal gland.

Abbreviations: IgG1, immunoglobulin G1; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; TCR, tissue cross-reactivity

13.1.4.2. Genetic Toxicology, Carcinogenicity, Reproductive Toxicology

REGN3470, REGN3471, and REGN3479 (REGN-EB3) are directed against an exogenous target (EBOV GP); therefore, in accordance with ICH Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (S6[R1], 2011), no toxicology studies other than a short duration repeat-dose study are typically required. mAbs are not expected to interact directly with DNA or other chromosomal material. The acute duration of dosing and MOA (binding to viral target) does not suggest any carcinogenic potential. The lack of findings in reproductive organs in the repeated-dose study and a MOA that is unlikely to impact reproduction suggests little-to-no potential for reproductive liability.

The initial tissue cross-reactivity study in fetal tissues identified cross-reactivity (membrane binding in the cervix, lung, eye, and thymus) that may affect the assessment for reproductive liability to embryos in utero. Regeneron stated that the membrane binding observed with REGN3471 and REGN3479 in this study was the result of nonspecific binding but did not provide direct evidence for this assertion.

Regeneron performed a follow-up exploratory competition study using excess REGN3471 and REGN3479 to block specific targets prior to performing a tissue cross-reactivity study in human fetal tissues of concern, except for the human cervix. Blockade of the specific binding of REGN3471-Bio and REGN3479-Bio (the tagged identification mAbs; see Study #REGN3479-TX-20001 for additional details on methods) by first applying REGN3471 or REGN3479 allowed for the assessment of binding independent of the complementarity-determining region. The preblock with REGN3471 or REGN3479 did not reduce the membrane or cytoplasmic staining of tissues identified in previous tissue cross-reactivity studies. These results indicate that the membrane and cytoplasmic staining observed with REGN3471-Bio and REGN3479-Bio in cryosections of select human adult/fetal tissues was likely nonspecific and of limited translational significance in vivo.

13.1.4.3. Other Toxicology/Specialized Studies

Immunogenicity

ADA were not assessed in the nonclinical program because development of ADA in monkeys is not predictive of immunogenicity in humans. The potential for ADA to affect exposure was, however, evaluated visually based on the concentration-time profiles in macaques. These assessments were performed as part of the PK studies described above. The concentration-time profiles of the following were characterized by a sudden, precipitous decline:

- 13% of the animals treated with REGN-EB3 in the single-dose cynomolgus monkey PK study.
- 21% of the animals treated with REGN-EB3 in the single-dose uninfected rhesus monkey PK study.
- 37% of the animals treated with REGN-EB3 in the single-dose infected rhesus PK study.
  - ADA impacted systemic concentrations by Day 26 (21 days posttreatment).
  - The impact on individual concentrations was similar for all three antibodies.

Concentrations considered to be impacted by ADA were excluded from mean concentration calculations and analysis.

ADA assays to detect anti-REGN3470, anti-REGN3471 and anti-REGN3479 antibodies in humans were developed late in development and after the conduct of the nonclinical program. Analysis of samples from the healthy volunteer trial (R3470-3471-3479-HV-1528-BA-01V1) yielded no positive results. These results were generally expected because REGN-EB-3 is a fully human mAb cocktail and the first-in-human study consisted of a single administration. Therefore, to confirm that the assays were accurate (as it was designed to target a 5% false positive rate), Regeneron tested samples from the cynomolgus monkey single-dose IV PK study (see the study on page 88). ADA analysis identified positive responses in postdose samples from two animals, both of which displayed increased clearance of REGN-EB3. One predose sample (false positive) was detected by the anti-REGN3471 ADA assay.

13.1.5. Excipients/Impurities/Degradants

No novel excipients or excipients in greater concentrations than previously approved are used in the manufacture of the final drug product and it contains no excipients of human or animal origin.

The qualification of actual and potential impurities that may arise during the manufacture and storage of REGN-EB3 drug product is described below. DP impurities are categorized into process and product impurities and may arise from:

- Raw materials
- DP manufacturing process
- REGN-EB3 degradation

Overall, the proposed specifications, or lack of specifications, are considered acceptable from a pharmacology/toxicology perspective. This conclusion is based on the use of a similar drug product formulation in nonclinical and clinical studies; the nature of this biologic product, which is given as a single dose; and a rabbit pyrogen test.

Drug Product Lot Comparison

A comparison of REGN-EB3 used across development is provided in Table 61. Toxicology studies were performed using (b) (4) 50 mg/mL drug product (b) (4) until the time of IV bolus injection. Clinical studies and the intended commercial formulation use a coformulated liquid in glass vials, which contains 50 mg/mL drug product refrigerated for IV administration.

Table 61. REGN-EB3 Drug Product Formulations for Nonclinical and Clinical Studies

	Toxicology DP	Clinical DP	Commercial DP
Storage conditions	(b) (4)	2 – 8 °C	2 – 8 °C
Protein concentration	(b) (4) or 50 mg/mL REGN-EB3 (16.7 mg/mL REGN3470, 16.7 mg/mL REGN3471, 16.7 mg/mL REGN3479)	50 mg/mL REGN-EB3 (16.7 mg/mL REGN3470, 16.7 mg/mL REGN3471, 16.7 mg/mL REGN3479)	50 mg/mL REGN-EB3 (16.7 mg/mL REGN3470, 16.7 mg/mL REGN3471, 16.7 mg/mL REGN3479)
histidine	10 mM	10 mM	10 mM
pH	6.0	6.0	6.0
Sucrose	10% (w/v)	10% (w/v)	10% (w/v)
Polysorbate 80	0.1% (w/v)	0.1% (w/v)	0.1% (w/v)
Primary container and closure	(b) (4) Glass vial with (b) (4) (b) (4) stopper	(b) (4) Glass vial with (b) (4) (b) (4) stopper	(b) (4) Glass vial with (b) (4) (b) (4) stopper

Source: eCtd loc. 2.3.P
Abbreviations: DP, drug product; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; TCR, tissue cross-reactivity; w/v, weight per volume

Process-Related Impurities

Potential process-related impurities originating from the DP manufacturing process and a summary of the control strategy for each is provided in Table 62. A rabbit pyrogen test was performed on three lots of REGN-EB3 DP. The lots were administered at the maximum dose of 150 mg/kg in rabbits. All of the tested lots were negative for the presence of pyrogens. See the product quality review for additional details on the DP impurities and the rabbit pyrogen test.

Table 62. Potential Process-Related Impurities

Impurity/ Substance	Source	Control Strategy
Microbial Contamination (Bioburden)	Bacterial or fungal contamination (viable)	(b) (4)
Endotoxin	Bacterial contamination (viable or nonviable)	
Particulate Matter	(b) (4)	
	Container closure	
Elemental Impurities	(b) (4) container, and closure	
Leachables	Disposable raw materials	
	Container and closure	

DP, drug product; (b) (4) ICH, International Council for Harmonisation; (b) (4) (b) (4)

Source: eCTD loc. 2.3.P

REGN-EB3 Related Impurities

Potential product-related impurities of REGN-EB3 DP are degradation products or modified REN-EB3 proteins that may have been inherent in the (b) (4) or formed during DP manufacturing and storage. The potential REGN-EB3 DP-related impurities and a control strategy for each is listed in Table 63.

Table 63. Potential REGN-EB3 Drug Product-Related Impurities

REGN-EB3 Variant	Primary Analytical Technique	Control Strategy
HMW Forms	(b) (4)	(b) (4)
LMW Forms		
Charge Variant Forms (including deamidated species)		
Oxidized Species	Not routinely tested	

(b) (4)

Source: eCTD loc. 2.3.P

13.1.6. Extractables/Leachables

As a result of the product quality review of leachables from storage of the DP, elevated elemental impurities (b) (4) were calculated to potentially be greater than the permissible daily exposure of (b) (4) However, the Applicant provided additional clarification concerning a dissolution step not factored into the initial review of product quality. (b) (4) Therefore, the level of elemental impurities is considered acceptable.

13.1.7. Referenced NDAs, BLAs, DMFs

Not applicable.

13.1.8. Individual Reviews of Studies Submitted to the NDA

Not applicable.

14. Clinical Pharmacology Assessment: Additional Information

14.1. In Vitro Studies

Not applicable.

14.2. In Vivo Studies

Study R3470-3471-3479-HV-1528 (Study 1528)

Study 1528 was a single ascending dose study to evaluate the safety and PK of REGNEB3 in healthy adults.

Methods

Dosing cohorts included placebo (n=6), 3 mg/kg (n=3), 15 mg/kg (n=3), 60 mg/kg (n=6) and 150 mg/kg (n=6). All doses were administered IV over a period of 2 hours (placebo, 3 mg/kg and 15 mg/kg) or 4 hours (60 mg/kg and 150 mg/kg).

Serum samples were collected predose and up to 169 days postdose for measurement of atoltivimab, maftivimab, and odesivimab concentrations. Serum samples were collected predose and on Day 85 and Day 169 for measurement of ADAs.

REGN-EB3 was measured using validated assays (Section III.14.4).

Results

Twenty-four subjects were enrolled (18 received REGN-EB3, 6 received placebo). The subjects were predominantly of white ethnicity (88%) and 58% of the subjects were female.

Concomitant medications were used by three subjects. Concomitant medications for two subjects included over-the-counter analgesics (Excedrin, ibuprofen, acetaminophen) for a duration of 1 day. The third subject used dexamethasone, cefdinir, and over-the-counter analgesics (ibuprofen, acetaminophen) with a maximum duration of 3 days. The use of these concomitant medications is not expected to impact the PK of REGN-EB3.

All protocol deviations were minor. These mostly concerned the timing of visits, blood draws, and/or PK samples relative to the protocol-specified time.

Exposures were found to be dose-proportional between 3 mg/kg and 150 mg/kg. Mean half-lives ranged from 20 to 32 days, typical for mAbs (Table 64). No ADAs were detected.

Table 64. PK Parameters in Uninfected Adults Administered a Single IV Dose of REGN-EB3

Parameter (units)	Total REGN3470 Mean (SD)				Total REGN3471 Mean (SD)				Total REGN3479 Mean (SD)			
	3 mg/kg IV (n=3)	15 mg/kg IV (n = 3)	60 mg/kg IV (n=6)	150 mg/kg IV (n=6)	3 mg/kg IV (n=3)	15 mg/kg IV (n=3)	60 mg/kg IV (n=6)	150 mg/kg IV (n=6)	3 mg/kg IV (n=3)	15 mg/kg IV (n=3)	60 mg/kg IV (n=6)	150 mg/kg IV (n=6)
C_{max} (mg/L)	25.7 (0.781)	155 (36.6)	543 (82.1)	1220 (101)	26.3 (0.586)	158 (34.5)	561 (81.3)	1260 (81.2)	27.0 (0.862)	159 (38.4)	564 (79.7)	1280 (68.0)
C_{max}/Dose (mg/L/mg)	0.408 (0.0116)	0.395 (0.0357)	0.379 (0.0241)	0.358 (0.0541)	0.418 (0.0239)	0.403 (0.0165)	0.392 (0.0255)	0.368 (0.0526)	0.428 (0.0275)	0.406 (0.0475)	0.394 (0.0193)	0.375 (0.0533)
AUC_{inf} (day*mg/L)	372 (47.9)	2060 (169)	7130 (1420)	17100 (4480)	586 (67.1)	2950 (491)	11300 (1950)	25600 (5040)	449 (58.3)	2300 (254)	8090 (1500)	18700 (4100)
AUC_{inf}/Dose (day/mg/L/mg)	5.91 (0.850)	5.40 (1.28)	5.02 (0.971)	5.05 (1.69)	9.29 (1.06)	7.75 (2.04)	7.98 (1.45)	7.53 (2.03)	7.13 (0.874)	6.04 (1.53)	5.71 (1.04)	5.52 (1.60)
$t_{1/2}$ (day)	22.7 (3.23)	20.2 (7.11)	22.3 (2.96)	21.2 (3.36)	31.7 (3.98)	26.7 (12.0)	27.4 (4.08)	25.3 (3.86)	25.4 (3.81)	22.0 (8.50)	23.9 (3.93)	22.3 (3.09)
CL (mL/day/kg)	2.72 (0.366)	2.44 (0.191)	2.9 (0.556)	3.08 (0.719)	1.72 (0.193)	1.73 (0.264)	1.82 (0.33)	2.02 (0.374)	2.25 (0.281)	2.19 (0.23)	2.54 (0.477)	2.78 (0.558)
V_{ss} (mL/kg)	67.9 (4.97)	56.3 (16.5)	55.4 (7.8)	58.2 (2.66)	66.2 (3.26)	55.2 (19.6)	53.9 (9.09)	56.0 (3.16)	64.0 (3.22)	55.8 (16.9)	56.1 (8.93)	57.6 (3.89)

Source: Study report, page 71
Abbreviations: AUC from time zero extrapolated to infinity; CL, total body clearance; C_{max}, peak concentration; IV, intravenous; n, number of subjects in subgroup; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; $t_{1/2}$, elimination half-life; V_{ss}, volume of distr bution at steady state

14.3. Comparison of PK in NHP and Humans

Studies

Study 1528 evaluated the PK of REGN-EB3 in uninfected humans administered a single dose of 3 to 150 mg/kg (Section III.14.4). Because target (GP or sGP) is not present in samples, free drug concentrations were measured.

Study R3479-PM-18034 (study 18034) evaluated the PK of REGN-EB3 in uninfected NHP. NHP received a single dose of 30 mg/kg (n=3), 100 mg/kg (n=6), 150 mg/kg (n=6) or 300 mg/kg (n=6). The duration of PK sampling was 85 days postdose. REGN-EB3 was measured using validated assays (Section III.14.4). As target (GP or sGP) is not present in the sample, free drug concentrations were measured.

Study R3479-PM-18140 (study 18140) evaluated the PK and PD of REGN-EB3 in infected rhesus macaques. NHPs were infected with Ebola virus on Day 0 and received a single dose of REGN-EB3 (30 mg/kg, 100 mg/kg, 150 mg/kg, or 300 mg/kg; n=4 to 5 per group) or vehicle on Day 5. Blood samples were collected through study Day 47 for determination of drug concentrations, sGP, and viral load. REGN-EB3 was measured using assays validated with uninfected NHP serum (Section III.14.4). As such, the effects of sGP and GP (i.e., interference) were not determined. According to the Applicant, the assay most likely does not detect drug bound to target, in which case free drug concentrations are measured, but no detailed information

has been provided to support their claim. The Pharm/Tox review team concluded that study 18140 was poorly conducted and that no definitive conclusions can be made from the results.

Comparison of PK

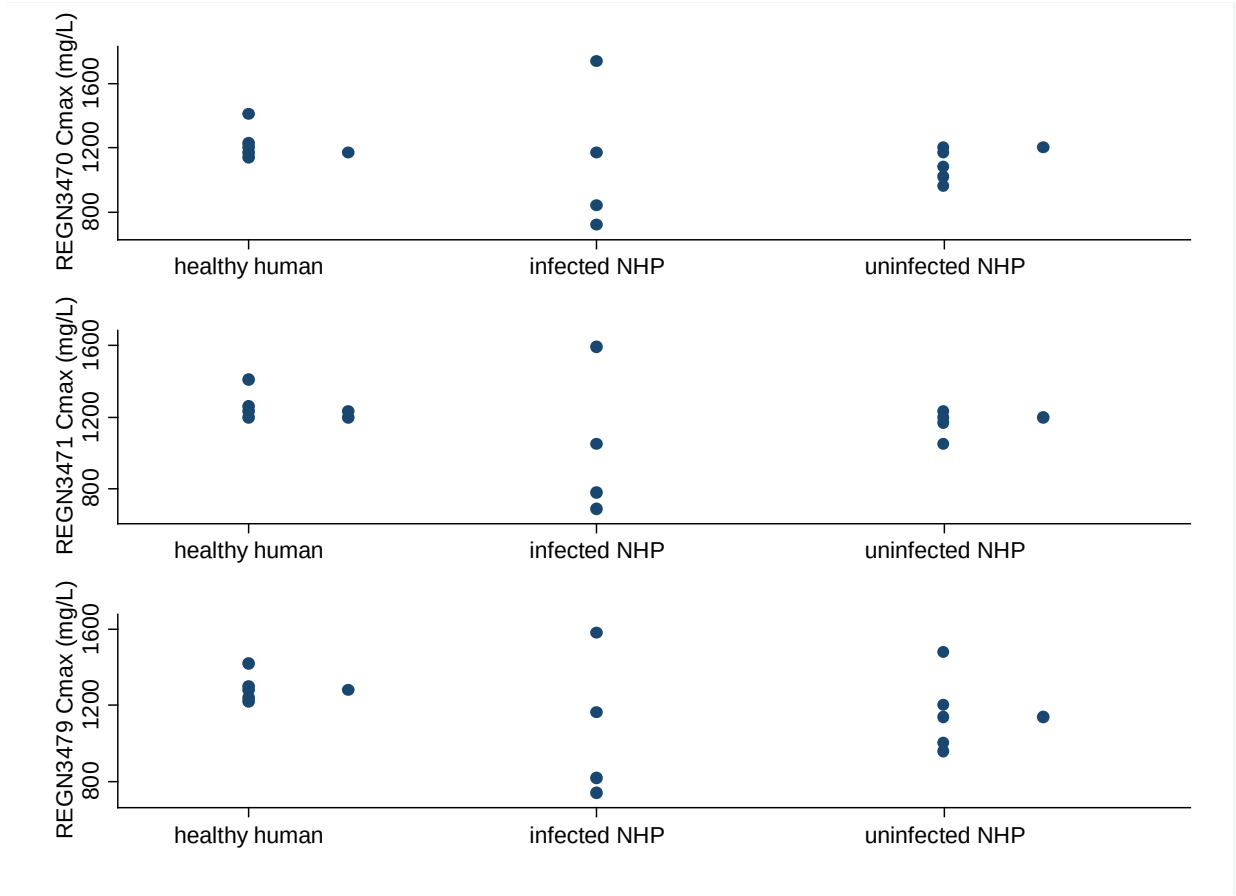
A single dose of 100 mg/kg was considered by the Applicant to be fully effective in NHP and a single dose of 150 mg/kg was evaluated in the PALM trial. Due to uncertainty as to the fully effective dose in NHP, our analyses focused upon comparison of exposures at a dose of 150 mg/kg in uninfected NHPs vs. infected NHPs vs. uninfected humans.

REGN-EB3 C_{max} values were slightly higher in uninfected human vs. uninfected NHPs. Much greater variability was observed in infected NHPs vs. other groups (Figure 10). REGN-EB3 AUC_{inf} values were highest in uninfected humans followed by uninfected NHPs then infected NHPs. Only one area under the concentration-time curve (AUC) value in infected NHPs was reliable (with full PK sampling to 43 days postdose); other values in infected NHPs were unreliable due to PK sampling ≤14 days postdose (Figure 11). REGN-EB3 half-life values were highest in uninfected humans followed by uninfected NHPs then infected NHPs. Only one half-life value in infected NHPs was reliable (with full PK sampling to 43 days postdose); other values in infected NHPs were unreliable due to PK sampling ≤14 days postdose (Figure 12).

Due to unreliable PK data in infected NHPs, the impact of infection on PK in NHPs administered 150 mg/kg cannot be determined.

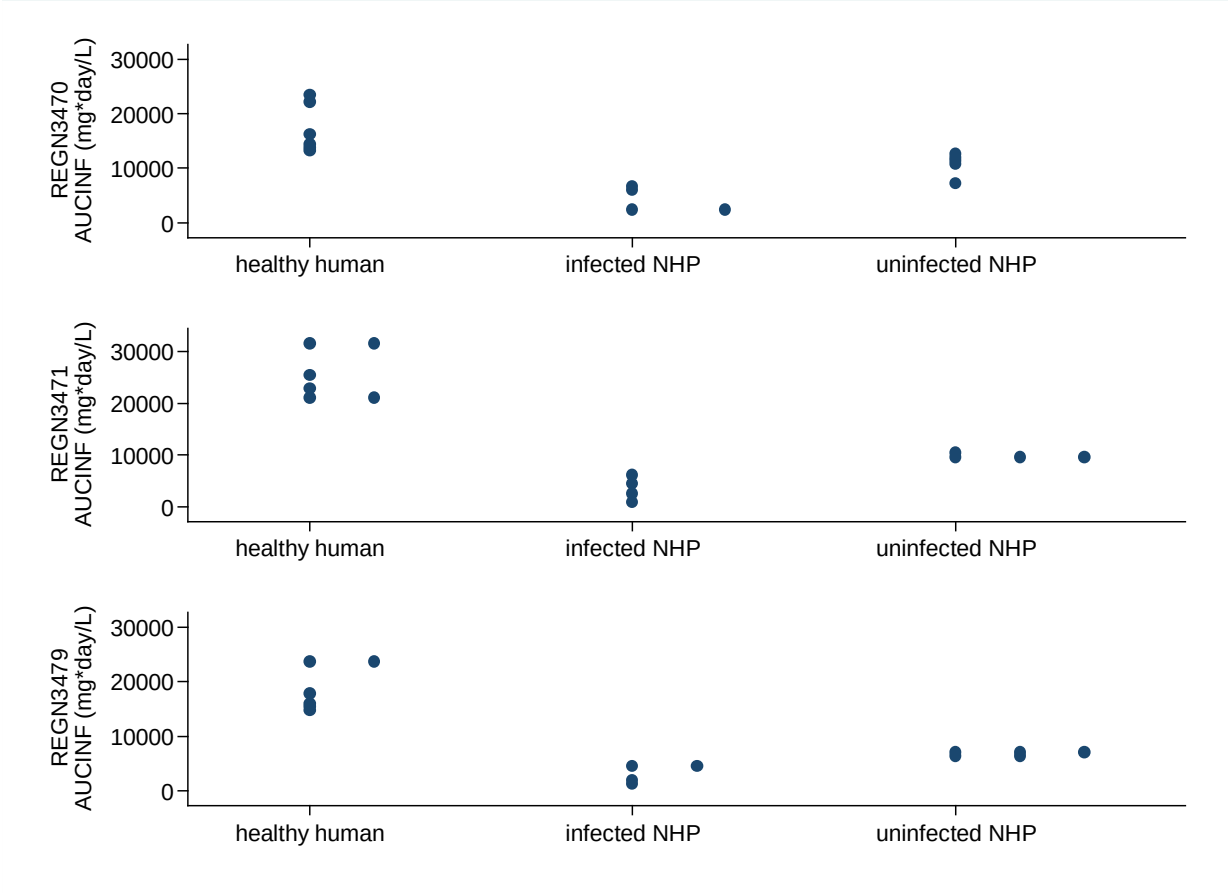
In conclusion, at 150 mg/kg, higher exposures were observed in uninfected humans vs. uninfected NHPs. Using a fully effective dose of either 100 mg/kg or 150 mg/kg in NHPs, exposures in humans administered 150 mg/kg would be anticipated to exceed exposures associated with administration of the fully effective dose to NHPs. With the assumption of a similar effect of infection on PK in NHPs and humans, the selection of 150 mg/kg for the PALM trial was reasonable based on the available data in NHPs and healthy subjects at the time of the decision. However, additional data obtained in the PALM trial as well as NHP studies suggest that 150 mg/kg may not be the optimal dose (Section II.6).

Figure 10. Comparison of C_{max} at 150 mg/kg Between NHPs and Humans



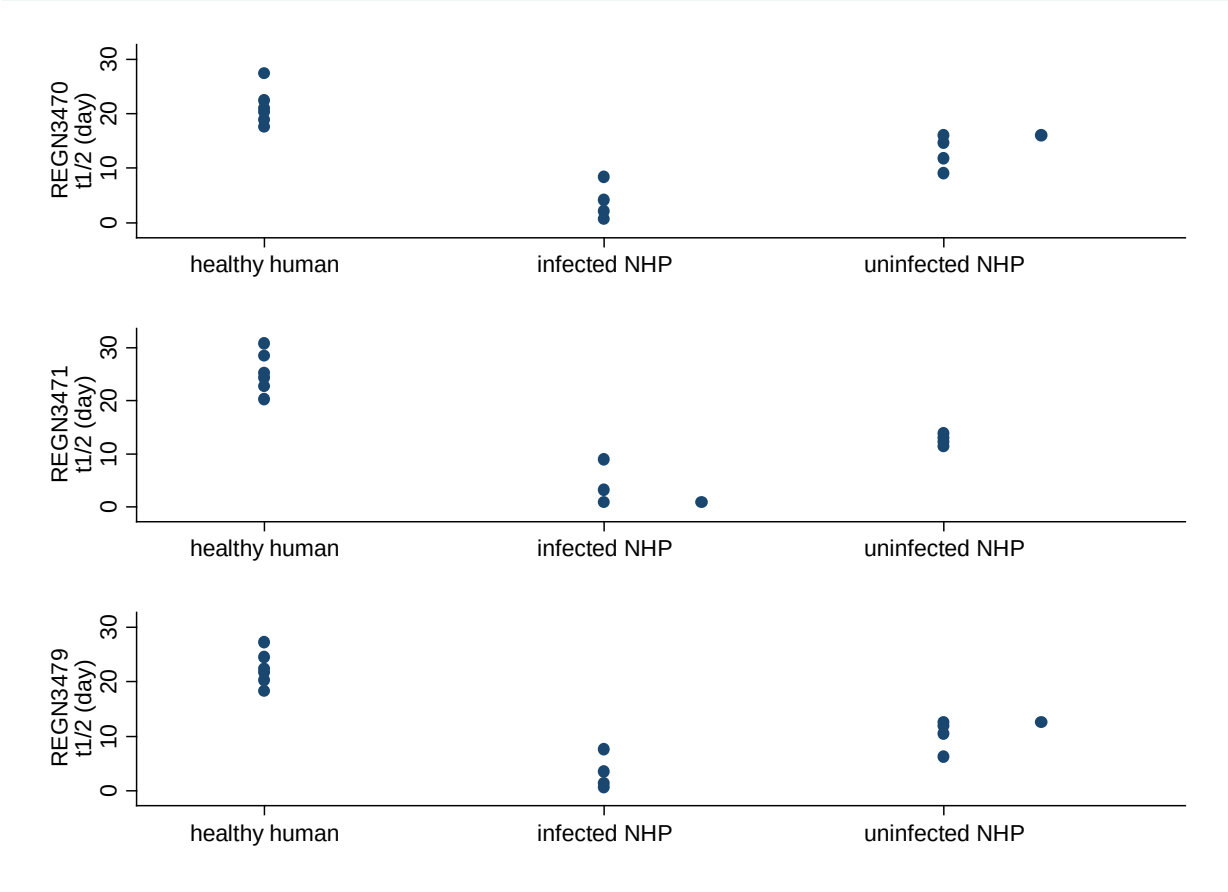
Source: Plotted by reviewer.
One value in uninfected NHPs was excluded due to suspected ADA starting with the end of infusion sample (5 minutes postdose).
Abbreviations: C_{max}, peak concentration; NHP, nonhuman primate; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Figure 11. Comparison of AUC_{inf} at 150 mg/kg Between NHPs and Humans



Source: Plotted by reviewer.
Two uninfected NHP values excluded due to ADA suspected by day 15 postdose
Abbreviations: AUC_{inf}, AUC from time zero extrapolated to infinity; NHP, nonhuman primate; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Figure 12. Comparison of Half-Lives at 150 mg/kg Between NHPs and Humans



Source: Plotted by the reviewer.
Two uninfected NHP values excluded due to ADA suspected by day 15 postdose.
Abbreviations: NHP, nonhuman primate; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; $t_{1/2}$, elimination half-life

14.4. Bioanalytical

Measurement of REGN-EB3 Using a Multiplexed Immunoassay in Serum of Uninfected Humans (Validation Report, Sample Analysis Report)

During validation and analysis of study 1528 samples, the accuracy and precision values for calibration and QC samples were within 15% (20% at the lower limit of quantification). The duration of stability at -20°C is 12 months and at -80°C is 24 months. Study samples were measured within the duration of stability (earliest sample collection in May 2016, samples measured in May 2017). One-hundred percent of incurred sample reanalysis samples passed (concentration within 30% of the original measurement). We consider the assays for measurement of REGN-EB3 in uninfected human serum to be validated.

Measurement of REGN-EB3 in Serum of Infected Humans

Not applicable. Drug concentrations were not measured from samples collected in the PALM trial.

Measurement of REGN-EB3 Using a Multiplexed Immunoassay in Serum of Uninfected NHPs (Validation Report, Sample Analysis Report on p. 93 of the Study Report)

During validation and analysis of study 18034 samples, the accuracy and precision values for calibration and QC samples were within 15% (20% at the lower limit of quantification). The duration of stability at -70°C is 12 months. Study samples were measured within the duration of stability (6 to 11 months after sample collection, Response to IR). Of the incurred sample reanalysis samples, 93 to 100% passed (concentration within 30% of the original measurement). We consider the assays for measurement of REGN-EB3 in uninfected human serum to be validated.

Measurement of REGN-EB3 in Serum of Infected NHPs

The study report states that the method used to measure REGN-EB3 in infected rhesus serum was based on the method used to measure REGN-EB3 in uninfected rhesus serum (page 1,636 for discussion of validation, page 1,561 for the sample analysis report). However, the method was validated using uninfected rhesus serum; thus, the interference by sGP or GP has not been determined. It has not been documented whether free vs. target-bound drug is measured or whether the presence of GP or sGP in the sample affects determination of REGN-EB3 concentrations (i.e., interference). Inadequate validation information was submitted.

15. Trial Design: Additional Information and Assessment

Note: The protocol synopsis was provided by the Applicant. Cross-references in this section are therefore not consistent with the remainder of the review.

1. Protocol Overview and Conduct

Applicant:	Regeneron Pharmaceuticals, Inc.
Drug Name:	Atoltivimab, maftivimab, and odesivimab-ebgn (also known as REGN-EB3 and REGN3470-3471-3479)
Indication:	Treatment of *Zaire ebolavirus* Infection
Protocol Title:	The PAmoja TuLinde Maisha (PALM) study: A Multicenter, Multi-Outbreak, Randomized, Controlled Safety and Efficacy Study of Investigational Therapeutics for the Treatment of Patients with Ebola Virus Disease
Source of Information:	1) PALM randomized controlled trial (RCT) Protocol Version 7.0 2) PALM RCT National Institute of Allergy and Infectious Diseases (NIAID) SAP version 2.0 3) Regeneron PALM RCT SAP (Original, November 26, 2019) 4) PALM RCT clinical study report (CSR)

Trial Identifiers	
Protocol Number:	19-I-0003
Clinical Phase:	Phase 2/3
EudraCT Number:	Not applicable
Other Codes:	Not applicable
IND Number:	125530
ClinicalTrial.gov identifier:	NCT03719586
Ethics:	(b) (4)
Trial Centers:	Beni, Butembo, Katwa, Mangina

1.1. Design

Planned duration of main phase:	Patient clinical status was monitored daily until discharge from the Ebola Treatment Unit (ETU) or death. Postdischarge study follow-up conducted on Day 58.
Planned duration of extension phase:	To be determined
Trial Status	Main Phase: Completed Ongoing: Extension phase started August 10, 2019
Date of Database lock:	January 17, 2020
Other Important Dates	November 20, 2018: PALM RCT initiated as group protocol (ZMapp, remdesivir, mAb114) December 12, 2018: PALM RCT was amended to add REGN-EB3 as a fourth group January 26, 2019: Enrollment into the REGN-EB3 group began

1.2. Objectives

The objectives of the protocol were based on the inclusion of 4 investigational therapeutics: ZMapp (control group), REGN-EB3, mAb114, remdesivir. For the purposes of the Regeneron Biologics Licensing Application, the analyses presented in the CSR and other submission documents focused on the assessment of effects of REGN-EB3, both in absolute terms and relative to ZMapp.

The extension phase is not included in this submission and thus the study design is not further summarized.

1.2.1 Primary Objective

The primary objective of this report was to compare 28-day mortality in patients with EVD who received REGN-EB3 with those who received ZMapp (control group).

1.2.2. Secondary Objective

- To evaluate safety and tolerability of REGN-EB3 relative to ZMapp.
- To compare changes in viral load over time between study groups.
- To assess the relationship between change in viral load over time and survival.
- To compare time to death of participants receiving REGN-EB3 and ZMapp.
- To compare time to discharge from ETU between participants receiving REGN-EB3 and ZMapp.
- To compare time to the first of 2-consecutive negative EBOV RT-PCR between participants receiving REGN-EB3 and ZMapp.
- Estimate and compare (vs ZMapp) the 28-day mortality rates for patients treated with REGN-EB3 in patients in key subgroups defined by baseline variables, including demographics, viral load (RT-PCR CT nucleoprotein [NP] gene targets [CtNP] or RT-PCR CT glycoprotein [GP] gene targets [CtGP]), age, pregnancy status, AST, ALT, creatinine and days since symptom onset (to the day of randomization).
- Estimate the effect of REGN-EB3 on key laboratory parameters, including but not limited to ALT, AST, and creatinine (including magnitude, time course of change) along with comparisons to changes in laboratory parameters in the ZMapp group of the study.

1.2.3. Exploratory Objectives

- Assess the relationship between baseline demographics, viral load (CtNP and/or CtGP), clinical laboratory assessments and days since symptom onset to randomization for patients who died within 24 hours and patients who lived for more than 24 hours.
- Explore relationships between baseline characteristics, including demographics, CtNP, CtGP, days since symptom onset to randomization, and laboratory parameters, and explore combinations of factors to predict outcome.
- Assess time course of changes in viral load (CtNP and CtGP) and laboratory values, between REGN-EB3 and ZMapp group, in patients who survived and in patients who died.

1.3. Selection of Trial Population

In addition to the inclusion and exclusion criteria provided below, the following key points about pregnant women and children and neonates should be noted:

- Although a full understanding of the potential risks from the study medications to human fetuses was lacking, given the mortality associated with Ebola virus infection and the likelihood that there is a greater risk to the fetus from severe EVD than from the study medications themselves, pregnant women were permitted entry into the study.
- Although study medications had only been tested in limited fashion, or not at all, in children, children of any age were eligible for enrollment given the likelihood that untreated Ebola infection may pose greater risk than exposure to the study medications. Neonates (defined as ≤7 days old) born to a mother who was RT-PCR positive for acute Ebola virus were presumed to be RT-PCR positive for acute Ebola virus at delivery and

were eligible for enrollment even prior to RT-PCR confirmation (i.e. obtaining those results could pose unnecessary delay).

1.3.1. Key Inclusion Criteria

- Males or females of any age with documented positive RT-PCR (Cepheid assay) for acute Ebola virus infection within 3 days prior to enrollment and who had symptoms of any duration (see special provision for neonates above).
- Willingness of study participant to accept randomization to any assigned treatment group.
- All males and females of childbearing potential must have been willing to use effective methods of contraception, from time of enrollment until Day 58 of study.
- Must have agreed not to enroll in another study of an investigational agent prior to completion of Day 28 of study.
- Ability to provide informed consent personally, or by a legally acceptable representative if the patient was unable to do so.

1.3.2. Key Exclusion Criteria

- Patients who, in the judgment of the investigator, were unlikely or unable to comply with the requirements of this protocol through Day 28.
- Prior treatment with any investigational antiviral drug therapy against Ebola virus infection within 5 half-lives or 30 days, whichever was longer, prior to enrollment. (Patients who received an experimental [or, in future, potentially a licensed] immunization against EBOV remained eligible.)

1.4. Hypotheses

The primary hypothesis was that the 28-day mortality rate of the REGN-EB3 will be lower than that of the control arm (ZMapp).

1.5. Treatment Groups

The PALM RCT CSR presents the results for the ZMapp and REGN-EB-3 treatment groups only.

Patients were randomized 1:1:1:1 into 1 of 4 treatment groups (each given with oSOC):

- ZMapp, 3 doses of 50 mg/kg of body weight administered by IV infusion every third day beginning on day 1, or
- REGN-EB3 at 150 mg/kg of body weight administered IV on day 1 as a single infusion, or
- mAb114, at 50 mg/kg of body weight administered IV on day 1 as a single infusion, or
- remdesivir, administered IV with a loading dose on day 1 (200 mg for adults and pediatric patients with body weight ≥40 kg and for pediatric patients weighing <40 kg 1 loading dose of remdesivir 5 mg/kg) followed by 9 to 13 days of once-daily maintenance dosing starting on day 2 and extending through day 10 to 14 (100 mg for adults and pediatric patients with body weight ≥40 kg and for pediatric patients weighing <40 kg remdesivir 2.5 mg/kg)

1.6. Endpoints and Definitions

1.6.1. Primary Efficacy Endpoint

The primary endpoint was 28-day mortality.

1.6.2. Secondary Efficacy Endpoints

The secondary efficacy endpoints were:

- Time to first negative EBOV RT-PCR in blood.
- Time to the first of 2-consecutive negative EBOV RT-PCRs in blood.
- RT-PCR over time (CtNP, CtGP).
- Time to discharge from ETU.
- Relationship among 28-day mortality, demographics, baseline viral load (CtNP and/or CtGP) and chemistry values according to duration since symptom onset.
- Death distributed by study day.
- Chemistry values (AST, ALT, AST/ALT ratio, creatinine) by study day.
- Viral load, expressed as $\log_{10}$ RNA viral copies per mL, over time, derived using the standard curve in (Pinsky et al. 2015):
  - Viral load using CtNP: (CtNP − 46.11)/(-3.44)
  - Viral load using CtGP: (CtGP − 51.45)/(-3.87)

1.6.3. Exploratory Efficacy Endpoints

The exploratory efficacy endpoints were:

- Relationship between 28-day mortality and key baseline characteristics, such as baseline viral load (CtNP and/or CtGP), chemistry values, and days since symptom onset to randomization, separated by early death (death within 24 hours) or late death (live at least 24 hours).
- Changes in viral load (CtNP and/or CtGP) and laboratory variables from baseline over time.

1.6.4. Safety Endpoints

The following endpoints were used in the assessment of safety:

- Incidence of serious adverse events (SAEs)
- Incidence of infusion-related adverse events (AEs)

1.7. Interim Analysis

Interim monitoring was prespecified in the protocol, to introduce new arms and allow early stopping for futility, efficacy, or safety.

Per protocol, interim monitoring used symmetric upper and lower boundaries for comparisons of a given arm to the control. The O'Brien-Fleming alpha-spending procedure will truncate boundaries at a one-sided type I error rate of 0.001. Four interim looks (including the final analysis) were planned, roughly corresponding to endpoint data from 33, 65, 100 and full enrollment (170 REGN-EB3 and 185 in each of the other 3 arms). The upper boundaries for the z-scores at these looks are 3.09, 3.09, 3.09, and 1.98. The protocol acknowledged that the timing of analyses might change depending on the size of the outbreak.

On 09 August 2019, the data safety monitoring board (DSMB) recommended stopping the PALM RCT before the planned enrollment (725 patients) was met and also recommended the Extension Phase commence with only REGN-EB3 and mAb114. These recommendations were based on interim analysis of 499 participants enrolled into the PALM RCT with at least 10 days of follow-up, which revealed that REGN-EB3 crossed prespecified boundary for efficacy over ZMapp. Mortality rates in the REGN-EB3 and mAb114 treatment groups were similar and both were lower than ZMapp (control group) and remdesivir groups. Thus, the DSMB recommended that the PALM RCT continue into an Extension Phase and randomize patients to either REGN-EB3 or mAb114 to evaluate safety.

Since the study stopped early, after the fourth interim analysis, the final assessment of significance was conservatively made using the 5th interim monitoring boundary. Thus, a p-value<0.028 (2-sided) for the comparison of REGN-EB3 vs. ZMapp at the final analysis was required to claim statistical significance for primary endpoint.

1.8. Data and Safety Monitoring Committee

An independent DSMB with international representation of the host countries participating in the study reviewed the study no less frequently than twice a year. The DSMB convened additional reviews as necessary, dependent on the rate of patient accrual. The DSMB reviewed the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All SAEs, all unanticipated problems, and all IND Safety Reports were reported by the Data Coordinating Center to the DSMB at the same time they were submitted to the IRB or IND Sponsor. The Principal Investigator submitted the DSMB's written summary open reports with the DSMB's recommendations to the IRB. A specific DSMB charter was put in place establishing the roles and responsibilities of members after review and approval by the Study Steering Committee (Mulangu et al. 2019).

The DSMB monitored safety, efficacy, and quality of study conduct measures closely throughout the study and may have pause enrollment in the event of unanticipated study-related deaths or SAEs that were considered study-related.

The DSMB also reviewed the completeness of follow-up and other aspects of study conduct.

After each meeting they recommended that the study be continued as planned, modified, or terminated.

1.9. Endpoint Adjudication Committee

Not applicable.

1.10. Sample Size Considerations

1.10.1. Sample Size Assumptions

The study initially targeted 125 patients per group based on an expected 28-day mortality rate of 30% in the ZMapp group, with a 50% relative reduction in the experimental treatment (i.e., rate of 15%). On 17 Jul 2019, a letter of amendment was submitted (b) (4) requesting to enlarge the sample size to 725 to increase power and allow for a smaller, clinically meaningful treatment effect than the original assumed 50% decrease in mortality from 30% (control) to 15% (new investigational product). Since REGN-EB3 was not included until amendment 3, the planned enrollment was 170 on REGN-EB3 vs 185 in each of the other treatment groups.

1.10.2. Rationale for NI Margin

Not applicable as this was a superiority study design.

1.10.3. Response Rate Assumptions

Refer to Section 1.10.1.

1.11. Analysis Population and Time Point Description

1.11.1. Analysis Populations

Intention to Treat (ITT) Concurrent Analysis Set

The ITT Concurrent population includes all randomized patients when REGN-EB3 and ZMapp were both concurrently eligible to be randomized treatments (i.e. after the implementation of the Amendment 3 of the protocol, on January 26, 2019, and excluding patients when either drug was not available). Patients are analyzed based on the assigned randomized treatment. The ITT Concurrent population is the primary analysis set for all efficacy endpoints.

ITT Overall Analysis Set

The ITT Overall population includes all randomized patients to either ZMapp or REGN-EB3. Patients are analyzed based on the first assigned randomized treatment. This population is included for a sensitivity analysis of the primary endpoint. Any discrepancies of primary analysis results among different populations will be explored.

ITT Amendment 3 Analysis Set

The ITT Amendment 3 population includes all randomized patients after the introduction of REGN-EB3 into the study (i.e. after the implementation of the Amendment 3 of the protocol, on January 26, 2019). Patients are analyzed based on the first assigned randomized treatment. This population is included for a sensitivity analysis of the primary endpoint.

The All Patients Treated (APT) Analysis Set

The APT population is a subset of ITT Amendment 3 analysis set. Patients who are confirmed not to be treated are excluded from the APT. Patients are analyzed based on the final assigned randomized treatment. The APT population is included for a sensitivity analysis of the primary endpoint.

The Safety Analysis Set (SAF)

The SAF population includes all patients who received either ZMapp or REGN-EB3 and will be analyzed as treated. Safety data for patients who received two therapies will be listed. The SAF population will be used for all safety analyses.

1.11.2. Time Point Description

The primary endpoint was 28-day mortality. Patients were followed to Day 58. Viral load measurements were collected at admission to the ETU and on days 1, 2, 3, 4, 5, 6, 8, 10, 14, and 28. Follow up viral load measurements were not systematically provided.

1.12. Analysis Description

1.12.1. Primary Efficacy Analysis Description

The primary efficacy analysis of 28-day mortality rate was compared between REGN-EB3 and ZMapp using Boschloo's test for participants who were concurrently randomized (ITT Concurrent analysis set). The 2-sided p-value was obtained by the Boschloo's test, and statistical significance was claimed if the 2-sided p-value was less than the monitoring boundary allocating a total type I error rate of 0.05 across interim analyses. Corresponding 95% confidence intervals were calculated.

For the overall study, the type I error rate was controlled at the 5% level. Interim monitoring boundaries were established using a truncated O'Brien-Fleming boundary. Since the study stopped early, after the fourth interim analysis, the final assessment of significance was conservatively made using the 5th interim monitoring boundary. Thus, a p-value<0.028 (2-sided) for the comparison of REGN-EB3 vs. ZMapp at the final analysis was required to claim statistical significance for primary endpoint. Missing value for 28-day mortality was imputed as death.

1.12.2. Sensitivity and Supportive Statistical Analyses Description

Sensitivity analyses were performed using the same statistical method in different analysis sets, including the ITT Overall analysis set, the ITT Amendment 3 analysis set and the APT analysis set. In addition, analyses using Mantel-Haenszel test statistic and risk difference were provided, with strata defined by site and baseline CtNP category (≤22 or >22) within the ITT Concurrent analysis set.

Descriptive analyses and Boschloo's test were performed on the primary endpoint (28-day mortality) to summarize the treatment effects across subpopulations defined by baseline factors.

Subgroup analyses were performed by age, sex, site, baseline CtNP, days from symptom onset to randomization, pregnancy, concomitant diagnosis of malaria, rVSV-ZEBOV vaccine, ALT, AST, and creatinine.

Descriptive statistics were provided to summarize death for patients who died within 1 day after randomization and patients who lived more than 1 day after randomization, based on patients' baseline characteristics, such as baseline CtNP, days from symptom onset to randomization and baseline chemistry values. Figures exploring the relationship between baseline CtNP, days from symptoms onset to randomization and baseline chemistry values for early death and late death were also provided.

There was only a single primary comparison of interest, REGN-EB3 vs ZMapp. However, multiplicity adjustment for multiple interim looks were made and overall type I error rate was controlled at 0.05 2-sided level. Interim monitoring for efficacy used symmetric upper and lower boundaries, with truncated O'Brien-Fleming alpha-spending procedures and truncation corresponding to a 1-sided error rate of 0.001. For the purpose of the final analysis, the boundary of the fifth interim analysis was used to declare statistical significance ($p<0.028$). Nominal p-values were provided for secondary efficacy endpoints.

Differences in median days to viral clearance (first negative CtNP in blood) were tested using a Wilcoxon rank-sum test, imputing deaths prior to 28 days as worst ranks, with earlier deaths having a worse rank than later deaths.

1.12.3. Safety Analysis

A table and a listing of adverse reactions during or post infusion were provided. These events were not entered as AEs with start/stop dates and a causality assessment and are therefore summarized just by the frequency of occurrence. Given that relationship to study drug was not assessed and the confounding by the underlying EVD, Regeneron considers the data collected to be more appropriately defined as signs and symptoms

Per the PALM Safety Reporting SOP, only SAEs were systematically collected during the study.

Serious adverse events were summarized by treatment group. Tables and patient listings for SAEs were provided with information on patient ID, treatment received, days from last infusion prior to SAE event onset, days from randomization to SAE onset, SAE start and stop day, SAE (MedDRA system organ class [SOC] and preferred term [PT], and verbatim description), severity grade, relatedness to study drug, likely cause and outcome.

Clinical laboratory assessments were summarized by treatment group. No imputation was made for analyses of these analytes for safety. Chemistry values were reviewed by NIAID at the screening visit. Optional procedures data including optional laboratory data were not cleaned or queried and were raw data. Potentially clinically significant values were not summarized for this study given the nature of Ebola virus disease.

A listing of vital signs was provided. No imputation was made for these parameters as safety variables. Vital sign data were not cleaned or queried and were raw data.

1.12.3.1. Viral Genotyping/Phenotyping Analyses

Not Applicable.

1.12.3.2. Pharmacokinetic Analyses

No pharmacokinetic analyses were conducted for this study.

1.12.3.3. Pharmacokinetic/Pharmacodynamic Analyses

Not Applicable.

1.12.3.4. Health Outcomes Analyses

Not Applicable.

1.12.4. Changes in Conduct of the Study or Planned Analyses

1.12.4.1 Changes to the Conduct of the Study

Protocol:

The PALM RCT study protocol was originally approved on 24 Sep 2018 (version 1.0) with the study beginning in November 2018. There were 6 amendments to the study protocol. REGN-EB3 was added in Version 3.0 of the protocol, and therefore, Regeneron only has access to versions 3.0 and later. Relevant changes from Versions 3.0 to 5.0 are summarized below. Versions 6.0 and 7.0 pertain to the Extension Phase, for which data are not presented in this report.

Version 3.0, 12 Dec 2018

- REGN-EB3 was added as the fourth investigational group of the PALM RCT based on the recommendations of an Ad-Hoc Consultation on Clinical Trials for Ebola Therapeutics, convened by the WHO in Geneva.
- Randomization was updated to occur on a 1:1:1:1 basis to the four study groups and the study sample size was increased to 500 patients to accommodate the addition of a fourth group.
- ICF was modified accordingly.

Version 3.1, 09 Jul 2019

- Clarified sample /study size to address addition of REGN-EB3 group
    - N=545 (n=125 per group plus additional subjects enrolled prior to accommodate transition from version 2 to version 3 of the protocol with the delayed start addition of the REGN-EB3 group). Accrual ceiling of 550, in order to randomize 545 to study treatment.
- Updated Reporting Procedures to the IRB (Section 10.5)
    - Combined and updated Expedited Reporting to [redacted] (b) (4) (Section 10.5.1) and Annual Reporting to IRB (Section 10.5.2) into Annual Reporting to IRB (Section 10.5.1) to reflect [redacted] (b) (4)

Version 4.0, Not Available

- Version 3.1 was a letter of amendment that was subsequently renumbered to Version 4.0 per NIAID requirement. Since this was a clerical amendment, NIAID did not distribute Version 4.0

Version 5.0, 29 Jul 2019

- Increased sample size to up to 725 randomized patients.
- Added an Extension Phase that would accrue up to an additional 300 patients and was to begin upon what was presumed patients. The Extension Phase would be the 726th enrollment, to be continued until study outcomes had been released and a new revised treatment plan had been designed, approved, and implemented.

  - Added Section 14 Extension Phase
  - Updated Table 2 Schedule of Evaluations, Section 6.4 Power and Sample Size, Section 6.5 Interim monitoring
  - Added Appendix G Sample Informed Consent and Assent for the Extension Phase.
- Added paragraph in Section 1.1.3 describing security challenges due to terrorists.
- ICF was modified accordingly.

DSMB recommendation:

The DSMB recommended stopping the PALM RCT before the planned enrollment (725 patients) was met and also recommended the Extension Phase commence with only REGN-EB3 and mAb114 (protocol Version 6.0). These recommendations were based on interim analysis of 499 participants enrolled into the PALM RCT with at least 10 days of follow-up, which revealed that REGN-EB3 crossed an early monitoring boundary for efficacy over ZMapp. Of 673 patients enrolled up to 09 Aug 2019, included in analyses, 290 died within 28 days, giving an overall mortality rate of 0.43; by baseline viral load, mortality rate was 0.19 (lower viral load, CtNP >22) and 0.76 (higher viral load, CtNP ≤22). The final PALM RCT cohort included 681 participants, the number enrolled up until the DSMB recommendation.

ETU issues:

Functionality of ETUs during the study were affected by the following:

Unanticipated Problem -Fire and Destruction

- Fire and destruction at international research sites
  - Katwa (24 Feb 2019): study team attacked, and study supplies destroyed
  - Butembo (27 Feb 2019): Fire, building destruction, and major material damage
- Led to temporary cessation of study enrollment but continuation of treatment of patients (with treatment transferred from the Beni site). Future patients to be enrolled in a location that would provide greater protection from attacks.
- Médecins Sans Frontiéres (MSF), which was providing staffing for these facilities did not engage after these events

Unavailability of ZMapp or REGN-EB3 at Site Beni

- ZMapp was quarantined between 23 Jan and 04 Feb 2019
- Delayed shipment of ZMapp leading to drug shortage on 28 Mar 2019
- REGN-EB3 was not immediately available from 10:30am to 1:40pm on 02 May 2019

1.12.4.2 Changes to the Planned Analyses

Regeneron generated a SAP to supplement the NIAID SAP. While the NIAID SAP included analyses for all 4 treatment groups, the Regeneron SAP included only analyses relevant to REGN-

EB3; therefore, comparisons to ZMapp were performed but analyses on mAb114 and remdesivir were not performed. Changes in the analyses from the Regeneron SAP include:

- A listing of patients who were switched to REGN-EB3 from ZMapp or remdesivir after 09 Aug 2019 was added
- Early death was originally defined as "patients who died within 24 hours after randomization" and was changed to "patients who died ≤1 day from randomization"
- SAEs were planned to be summarized by demographic and other patient characteristics. Based on the small number of SAEs, these analyses were not performed
- Subgroup analyses for age <1 month and for age ≥1month and <1 year were combined as 1 subgroup analysis: age <1 year, because of small sample size for the subgroup

16. Efficacy Assessment Additional Information and Assessment

16.1. Additional Analyses of Demographics

This section supplements the analyses and interpretation presented in Section II.6.3.1. The baseline predefined symptoms were similar between the two arms (Table 65). The symptoms with at least 20% reported at baseline were diarrhea, fever, abdominal pain, and vomiting.

Table 65. Baseline Predefined Symptoms, ITT Concurrent Population, PALM Trial

Subgroup	REGN-EB3	ZMapp	Total
ITT Concurrent			
N	154	153	307
Diarrhea			
Yes	98 (63.6%)	94 (61.4%)	192 (62.5%)
No	56 (36.4%)	59 (38.6%)	115 (37.5%)
Fever			
Yes	75 (48.7%)	58 (37.9%)	133 (43.3%)
No	79 (51.3%)	95 (62.1%)	174 (56.7%)
Abdominal pain			
Yes	49 (31.8%)	50 (32.7%)	99 (32.2%)
No	105 (68.2%)	103 (67.3%)	208 (67.8%)
Vomiting			
Yes	38 (24.7%)	46 (30.1%)	84 (27.4%)
No	116 (75.3%)	107 (69.9%)	223 (72.6%)
Conjunctival injection			
Yes	28 (18.2%)	28 (18.3%)	56 (18.2%)
No	126 (81.8%)	125 (81.7%)	251 (81.8%)
Shortness of breath/difficulty breathing			
Yes	23 (14.9%)	20 (13.1%)	43 (14.0%)
No	131 (85.1%)	133 (86.9%)	264 (86.0%)
Headache			
Yes	22 (14.3%)	27 (17.6%)	49 (16.0%)
No	132 (85.7%)	126 (82.4%)	258 (84.0%)
Hemorrhage			
Yes	21 (13.6%)	20 (13.1%)	41 (13.4%)
No	133 (86.4%)	133 (86.9%)	266 (86.6%)

Subgroup	REGN-EB3	ZMapp	Total
Cough			
Yes	16 (10.4%)	12 (7.8%)	28 (9.1%)
No	138 (89.6%)	141 (92.2%)	279 (90.9%)
Edema			
Yes	13 (8.4%)	3 (2.0%)	16 (5.2%)
No	141 (91.6%)	150 (98.0%)	291 (94.8%)
Mental state change			
Yes	7 (4.5%)	7 (4.6%)	14 (4.6%)
No	147 (95.5%)	146 (95.4%)	293 (95.4%)
Convulsions			
Yes	2 (1.3%)	4 (2.6%)	6 (2.0%)
No	152 (98.7%)	149 (97.4%)	301 (98.0%)
Hiccups			
Yes	2 (1.3%)	3 (2.0%)	5 (1.6%)
No	152 (98.7%)	150 (98.0%)	302 (98.4%)
Hearing loss			
Yes	1 (0.6%)	1 (0.7%)	2 (0.7%)
No	153 (99.4%)	152 (99.3%)	305 (99.3%)
Rash			
Yes	1 (0.6%)	5 (3.3%)	6 (2.0%)
No	153 (99.4%)	148 (96.7%)	301 (98.0%)
Vision loss			
Yes	1 (0.6%)	0 (0%)	1 (0.3%)
No	153 (99.4%)	153 (100.0%)	306 (99.7%)
Other symptoms			
Yes	82 (53.2%)	61 (39.9%)	143 (46.6%)
No	72 (46.8%)	92 (60.1%)	164 (53.4%)

Source: from Statistical reviewer, ADSL and SAS software used
Abbreviations: ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha

16.2. Additional Analyses for the Primary Efficacy Endpoint

This section supplements the analyses and interpretation presented in Section II.6.3.2. Detailed information on the subjects excluded from the intention-to-treat (ITT) concurrent analysis population and the two ITT Concurrent Sensitivity analysis populations is presented here.

16.2.1. Subjects Excluded From the ITT Concurrent Population for the Primary Efficacy Analysis

The drug-shortage periods occurred during the PALM trial are listed in Table 66. Six subjects, (b) (6) who received either REGN-EB3 or ZMapp when some investigational drugs were not available were excluded from the ITT Concurrent population. Three subjects, (b) (6) were rerandomized. Subject (b) (6) was randomized to the ZMapp arm during a shipment delay. This subject was rerandomized to ZMapp again. Because the site decided to wait for the assigned drug, Subject (b) (6) was included in the ITT Concurrent population. A summary of how these subjects differed between inclusion in each analysis population are provided in Table 67. Only subjects who received either REGN-EB3 or ZMapp are considered in the BLA.

Table 66. Drug Shortage Periods in the PALM Trial

Drug Shortage Period	Drug Not Available	Subject ID	Original Randomized Treatment Assignment	Treatment Assignment from Rerandomization	Actual Treatment Received
1/23/2019 to 2/4/2019	ZMapp	(b) (6)	REGN-EB3	—	REGN-EB3
			ZMapp	REGN-EB3	REGN-EB3
			REGN-EB3	—	REGN-EB3
3/28/2019	ZMapp		REGN-EB3	—	REGN-EB3
			REGN-EB3	—	REGN-EB3
			ZMapp	ZMapp	ZMapp
5/2/2019 10:30 AM to 1:40 PM	REGN-EB3		REGN-EB3	ZMapp	ZMapp

Source: reviewer analysis of the Randomization Quality Control Report, Data Handling Report, Listing 16.1.7.1 and DS dataset.
Abbreviations: PALM, PAmoja TuLinde Maisha

Table 67. Subjects Who Differed Between Analysis Populations

Subject ID	Final Randomized Assignment	Actual Treatment Received	ITT Concurrent	APT	Safety
(b) (6)	REGN-EB3	REGN-EB3	No	Yes	Yes
	REGN-EB3	REGN-EB3	No	Yes	Yes
	REGN-EB3	REGN-EB3	No	Yes	Yes
	REGN-EB3	REGN-EB3	No	Yes	Yes
	REGN-EB3	REGN-EB3	No	Yes	Yes
	ZMapp	ZMapp	Yes	Yes	Yes
	ZMapp	ZMapp	No	Yes	Yes

Source: adapted from the Applicant's table from Listing 16.2.3.1.
Abbreviations: APT, all patients treated; ITT, intention-to-treat

16.2.2. Sensitivity Analysis Populations for Primary Efficacy Endpoint Analyses

Two ITT Concurrent Sensitivity analysis populations were generated by the reviewer. The first, ITT Concurrent Sensitivity_1, is the ITT Concurrent with exclusion of subject (b) (6) who had a false-positive EVD infection at screening and was administered REGN-EB3.

The second, ITT Concurrent Sensitivity 2, the ITT Concurrent with exclusion of six subjects, (b) (6) who received ZMapp and were rerandomized to receive either REGN-EB3 or mAb114 after the trial was stopped and into the extension phase, from the ITT concurrent population.

Table 68. Eleven Subjects Who Were Rerandomized After Cessation of the PALM Trial

Subject ID	Original Randomized	Rerandomization After August 9, 2019 DSMB Decision	Included in Analysis Population Under Treatment		
			ITT Overall	ITT Concurrent	ITT Concurrent Sensitivity_2
(b) (6)	ZMapp	REGN-EB3	ZMapp	ZMapp	No
	Remdesivir	mAb114	No	No	No
	ZMapp	mAb114	ZMapp	ZMapp	No
	mAb114	REGN-EB3	No	No	No
	ZMapp	mAb114	ZMapp	ZMapp	No
	ZMapp	REGN-EB3	ZMapp	ZMapp	No
	Remdesivir	mAb114	No	No	No
	Remdesivir	mAb114	No	No	No
	ZMapp	mAb114	ZMapp	ZMapp	No
	Remdesivir	REGN-EB3	No	No	No
	ZMapp	mAb114	ZMapp	ZMapp	No

Source: Statistical reviewer; assembled from the materials submitted.
Abbreviations: DSMB, data safety monitoring board; ITT, intention-to-treat; mAb, monoclonal ant body; PALM, PAmoja TuLinde Maisha

The time from randomization to switch to a new treatment by rerandomization was 3 to 8 days for these six subjects, who received one to three doses of ZMapp (Table 69). Among those six subjects, only one died (on study Day 6); the others survived to Day 58.

Table 69. Subjects Who Received ZMapp But Were Rerandomized After the Interim Analysis, PALM Trial

Subject ID	Site	CtNP	Original Treatment	# of Doses Received	# of Days at Switch	New Treatment	Death?	End of Study Day
(b) (6)	Beni	>22	ZMapp	2	7	REGN-EB3		58
	Beni	>22	ZMapp	1	4	REGN-EB3		57
	Beni	>22	ZMapp	2	6	mAb114		59
	Beni	>22	ZMapp	2	5	mAb114		57
	Mangina	>22	ZMapp	3	8	mAb114		59
	Mangina	≤22	ZMapp	1	3	mAb114	Death	6

Source: Statistical reviewer; ADSL and SAS software used.
Abbreviations: CtNP, cycle-threshold nucleoprotein gene targets; mAb, monoclonal antibody; PALM, PAmoja TuLinde Maisha

16.3. Additional Analyses for Secondary Analyses

This section supplements the analyses and interpretation presented in Section II.6.3.2 with additional secondary efficacy analyses: discharge by study day and the Kaplan-Meier (KM) curve for the probability of survival.

Discharge from Ebola Treatment Unit by Study Day

A summary of death or discharge from the ETU is provided in Table 70. Subjects were discharged from the ETU as early as Day 4 and Day 6 for REGN-EB3 and ZMapp, respectively. Most subjects were discharged on Day 17 or Day 18. In total, by Day 28, 93 subjects (60%) in REGN-EB3 and 67 subjects (44%) in ZMapp were discharged from the ETU.

Table 70. Summary of Death and Discharge From ETU by Study Day, ITT Concurrent Population, PALM Trial

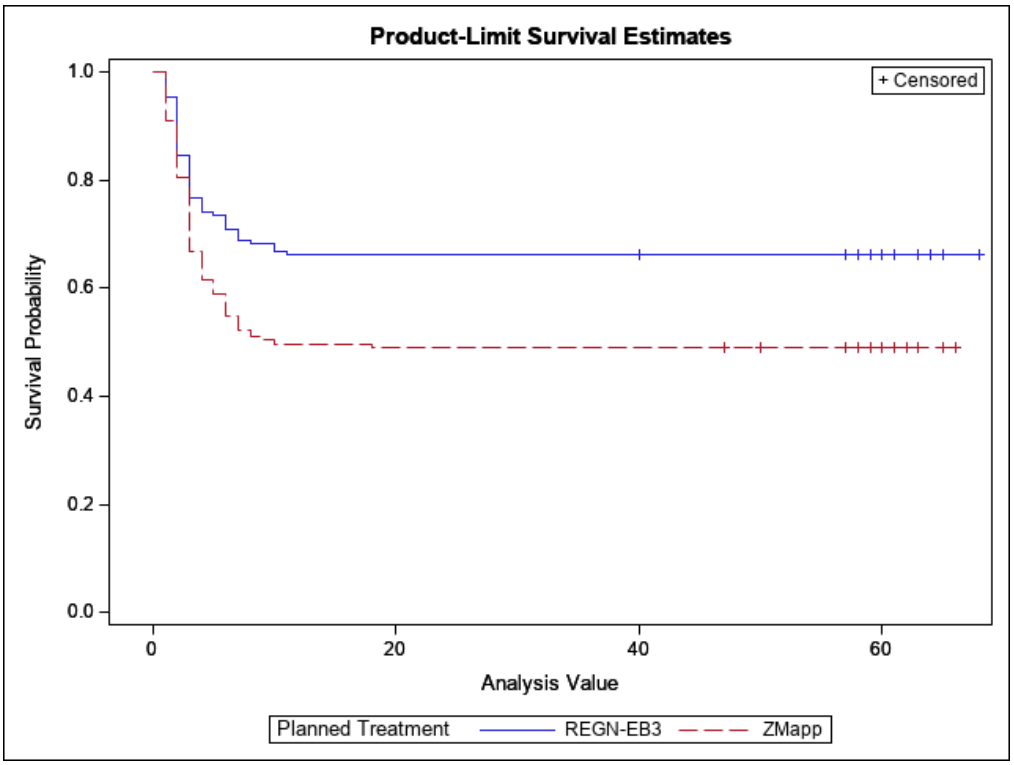
	REGN-EB3 (N=154)		ZMapp (N=153)	
	Death	Discharge from ETU	Death	Discharge from ETU
Total number of subjects	53	102	79	75
Study day of death or discharge, n (%)				
Day 1	7 (4.5)	0	14 (9.2)	0
Day 2	17 (11.0)	0	16 (10.5)	0
Day 3	12 (7.8)	0	21 (13.7)	0
Day 4	4 (2.6)	3 (1.9)	8 (5.2)	0
Day 5	1 (0.6)	2 (1.3)	4 (2.6)	0
Day 6	4 (2.6)	1 (0.6)	6 (3.9)	1 (0.7)
Day 7	3 (1.9)	1 (0.6)	4 (2.6)	1 (0.7)
Day 8	1 (0.6)	2 (1.3)	2 (1.3)	2 (1.3)
Day 9	0	4 (2.6)	1 (0.7)	1 (0.7)
Day 10	2 (1.3)	1 (0.6)	1 (0.7)	5 (3.3)
Day 11	1 (0.6)	3 (1.9)	0	1 (0.7)
Day 12	0	4 (2.6)	0	2 (1.3)
Day 13	0	3 (1.9)	0	2 (1.3)
Day 14	0	6 (3.9)	0	3 (2.0)
Day 15	0	11 (7.1)	0	9 (5.9)
Day 16	0	9 (5.8)	0	6 (3.9)
Day 17	0	8 (5.2)	0	12 (7.8)
Day 18	0	12 (7.8)	1 (0.7)	6 (3.9)
Day 19	0	5 (3.2)	0	2 (1.3)
Day 20	0	3 (1.9)	0	4 (2.6)
Day 21	0	3 (1.9)	0	1 (0.7)
Day 22	0	2 (1.3)	0	3 (2.0)
Day 23	0	5 (3.2)	0	1 (0.7)
Day 24	0	1 (0.6)	0	3 (2.0)
Day 25	0	2 (1.3)	0	2 (1.3)
Day 26	0	1 (0.6)	0	0
Day 27	0	1 (0.6)	0	0
Day 28	0	0	0	0
Days 29 to 31	0	3 (1.9)	0	1 (0.7)
Days 32 to 35	0	3 (1.9)	0	4 (2.6)
Day >35	1 (0.6)	3 (1.9)	1 (0.7)	3 (2.0)

Source: Statistical reviewer, ADSL and SAS software were used.
Note: if a subject was discharged on or before their Day 28 visit but died on a subsequent day, that subject may have been included in more than one column.
Abbreviations: ETU, Ebola treatment unit; ITT, intention-to-treat; N, number of subjects; n, number of subjects in subgroup; PALM, PAmoja TuLinde Maisha

KM Curve for the Probability of Survival

The KM curve for the probability of survival is shown in Figure 13. Because most deaths occurred within the first 3 days, the survival probability dropped sharply; thereafter, the survival probability in the REGN-EB3 arm remained higher than in the ZMapp arm. A log-rank test indicated a significant difference in the curve over time (p=0.0028).

Figure 13. Kaplan-Meier Curve for Survival, ITT Concurrent Population, PALM Trial



Source: from Statistical reviewer, ADTTE and SAS software used.
Abbreviations: ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha

16.4. Additional Subgroup Analyses for the Primary Efficacy Endpoint

This section supplements the analyses and interpretation presented in Section II.6.3.2. Of note, the sample sizes for many subgroups were small, which limits the ability to detect trends with certainty. Numerous subgroup analyses were conducted without any adjustment for the multiple analyses, which could result in spurious findings due to chance.

The treatment effect of REGN-EB3 compared to ZMapp appeared consistent across most baseline subgroups of age, gender, site, days from symptom onset to randomization, and other baseline factors analyzed although there were differences in the 28-day mortality rates. For example, the 28-day mortality rate for female subjects (58.8%) in the ZMapp arm was slightly higher than that for male subjects (42.5%) in the ZMapp arm, while female and male subjects had similar mortality rates in the REGN-EB3 arm. A similar pattern was evident for days from symptom onset to randomization ≤5 days vs. >5 days. A Forest plot with the same information is presented in Figure 14.

The impact of baseline viral load on the primary efficacy endpoint was discussed in Section II.6.4.2. For ALT, AST, and creatinine, the higher the baseline values over the upper limit of normal (ULN), the higher the 28-day mortality rate observed in both arms. In addition, the 28-day mortality rates in the REGN-EB3 arm were lower than those in the ZMapp arm across these subgroups.

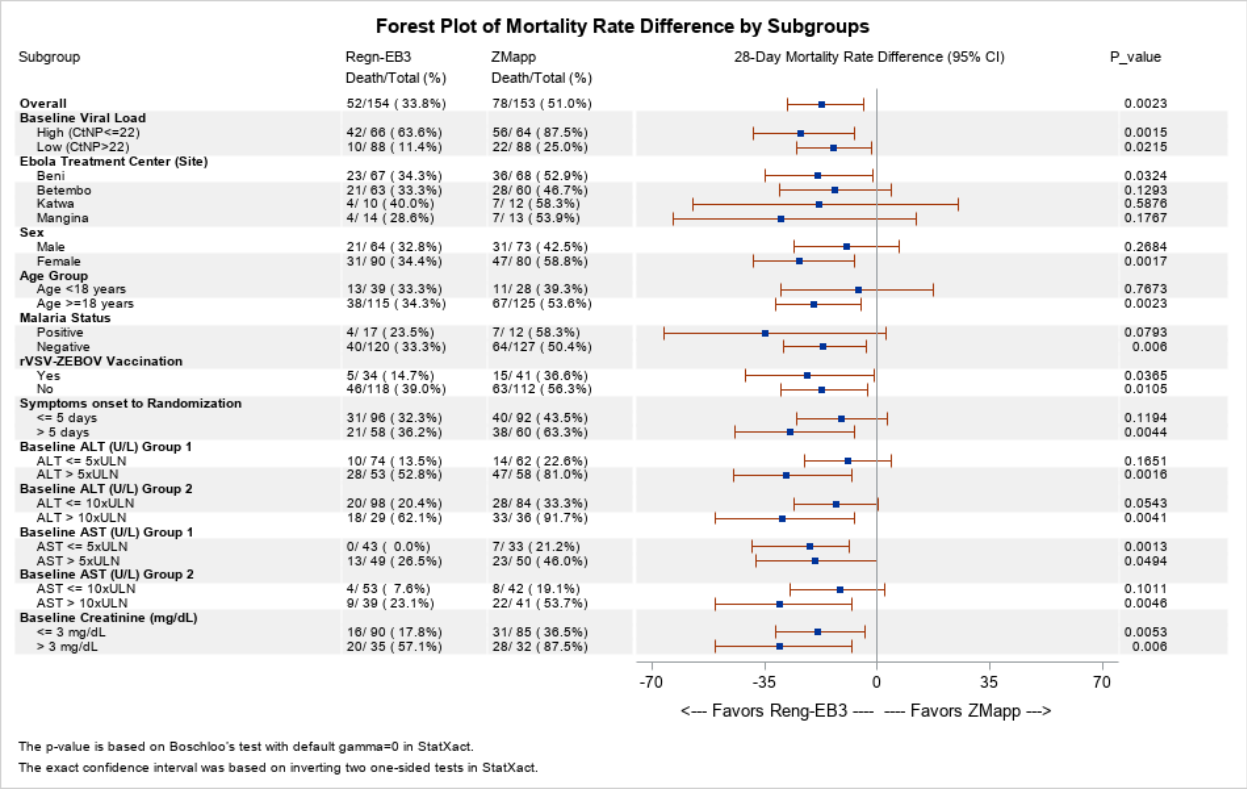
Table 71. Summary of 28-Day Mortality by Selected Baseline Factors, ITT Concurrent Population, PALM Trial

Population/ Subpopulation	REGN-EB3 (N=154) Death/Total (%)	ZMapp (N=153) Death/Total (%)	Rate Difference % (95% CI)[a]	Boschloo's Two-Sided P-Value[b]
ITT Concurrent	52/154 (33.8%)	78/153 (51.0%)	-17.2 (-28.0, -4.1)	0.0023
CtNP at Baseline				
CtNP ≤22	42/66 (63.6%)	56/64 (87.5%)	-23.9 (-38.5, -7.3)	0.0015
CtNP >22	10/88 (11.4%)	22/88 (25.0%)	-13.6 (-25.3, -1.9)	0.0215
Site or Ebola Treatment Center				
Beni	23/67 (34.3%)	36/68 (52.9%)	-18.6 (-34.9, -1.3)	0.0324
Butamben	21/63 (33.3%)	28/60 (46.7%)	-13.3 (-30.4, 4.4)	0.1293
Katwa	4/10 (40.0%)	7/12 (58.3%)	-18.3 (-57.2, 25.3)	0.5876
Mangina	4/14 (28.6%)	7/13 (53.9%)	-29.8 (-63.4, 12.1)	0.1767
Sex				
Male	21/64 (32.8%)	31/73 (42.5%)	-9.7 (-25.8, 6.9)	0.2684
Female	31/90 (34.4%)	47/80 (58.8%)	-24.3 (-38.6, -7.1)	0.0017
Age group				
<5 years	10/22 (45.5%)	6/15 (40.0%)	5.5 (-27.8, 37.0)	1.0
5, <12 years	1/9 (11.1%)	1/5 (20.0%)	-8.9 (-60.7, 36.0)	0.9999
12, <18 years	2/8 (25.0%)	4/8 (50.0%)	-25.0 (-67.8, 26.0)	0.3884
18, <50 years	30/93 (32.3%)	56/105 (53.3%)	-21.1 (-34.3, -5.2)	0.0032
50, <65 years	7/17 (41.2%)	9/18 (50.0%)	-8.8 (-42.5, 25.1)	0.7120
≥65 years	2/5 (40.0%)	2/2 (100.0%)	-60.0 (-94.7, 34.3)	0.2813
Age group				
<18 years	13/39 (33.3%)	11/28 (39.3%)	-6.0 (-29.8, 17.4)	0.7673
≥18 years	39/115 (33.9%)	67/125 (53.6%)	-19.7 (-31.8, -4.9)	0.0023
Malaria positive				
Yes	4/17 (23.5%)	7/12 (58.3%)	-34.8 (-66.3, 2.8)	0.0793
No	40/120 (33.3%)	64/127 (50.4%)	-17.1 (-29.2, -3.4)	0.0060
rVSV-ZEBOV Vaccination at baseline				
Yes	5/34 (14.7%)	15/41 (36.6%)	-21.9 (-41.2, -1.1)	0.0365
No	46/118 (39.0%)	63/112 (56.3%)	-17.3 (-29.8, -2.9)	0.0105
Unknown	1/2 (50%)	0/0		
Reported days before ETU admission for subjects with rVSV-ZEBOV Vaccination at baseline				
<10 days	2/20 (10.0%)	7/21 (33.3%)	-23.3 (-49.1, 3.3)	0.1173
≥10 days	3/14 (21.4%)	6/18 (33.3%)	-11.9 (-43.8, 21.9)	0.5996
Not Vaccinated	46/118 (39.0%)	63/112 (56.3%)	-17.3 (-29.8, -2.9)	0.0105
Pregnancy Test				
Positive	1/2 (50.0%)	1/4 (25.0%)	25.0 (-56.9, 89.0)	0.9688
Negative	20/66 (30.3%)	34/57 (59.7%)	-29.4 (-45.6, -8.7)	0.0014
Not applicable	30/82 (36.6%)	43/92 (46.7%)	-10.2 (-24.6, 5.0)	0.2006
Baseline ALT (U/L)				
≤5× ULN	10/74 (13.5%)	14/62 (22.6%)	-9.1 (-22.8, 4.1)	0.1651
>5× ULN	28/53 (52.8%)	47/58 (81.0%)	-28.2 (-44.7, -7.9)	0.0016
Baseline ALT (U/L)				
≤10× ULN	20/98 (20.4%)	28/84 (33.3%)	-12.9 (-26.1, 0.4)	0.0543
>10× ULN	18/29 (62.1%)	33/36 (91.7%)	-29.6 (-50.2, -7.3)	0.0041
Baseline AST (U/L)				
≤5× ULN	0/43 (0%)	7/33 (21.2%)	-21.2 (-39.1, -8.8)	0.0013
>5× ULN	13/49 (26.5%)	23/50 (46.0%)	-19.5 (-37.8, -0.2)	0.0494
Baseline AST (U/L)				
≤10× ULN	4/53 (7.6%)	8/42 (19.1%)	-11.5 (-27.2, 2.4)	0.1011
>10× ULN	9/39 (23.1%)	22/41 (53.7%)	-30.6 (-50.2, -8.0)	0.0046

Population/ Subpopulation	REGN-EB3 (N=154) Death/Total (%)	ZMapp (N=153) Death/Total (%)	Rate Difference % (95% CI)[a]	Boschloo's Two-Sided P-Value[b]
Baseline Creatinine (mg/dL)				
≤3 mg/dL	16/90 (17.8%)	31/85 (36.5%)	-18.7 (-31.7, -3.9)	0.0053
>3 mg/dL	20/35 (57.1%)	28/32 (87.5%)	-30.4 (-50.3, -7.9)	0.0060
Days from symptom onset to randomization by quartile				
< Q1 (3.0 days)	8/24 (33.3%)	10/25 (40.0%)	-6.7 (-33.6, 20.9)	0.7378
Q1, ≤Q2 (5.0)	23/72 (31.9%)	30/67 (44.8%)	-12.8 (-28.9, 3.8)	0.1431
Q2, ≤Q3 (7.0)	6/27 (22.2%)	22/29 (75.9%)	-53.6 (-73.6, -25.5)	0.0001
>Q3 (7.0 days)	15/31 (48.4%)	16/31 (51.6%)	-3.2 (-29.0, 22.9)	1.0
Days from symptom onset to randomization (median =5 days)				
≤5 days	31/96 (32.3%)	40/92 (43.5%)	-11.2 (-25.3, 2.9)	0.1194
>5 days	21/58 (36.2%)	38/60 (63.3%)	-27.1 (-44.1, -7.1)	0.0044

Source: Statistical reviewer; ADSL and SAS software were used.
[a] The exact confidence interval was based on inverting two one-sided tests in StatXact.
[b] P-value is based on Boschloo's test with default gamma=0 in StatXact.
Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; CtNP, cycle-threshold nucleoprotein gene targets; ETU, Ebola treatment unit; ITT, intention-to-treat; N, number of subjects; PALM, PAmoja TuLinde Maisha; rVSV, recombinant vesicular stomatitis virus; ULN, upper limit of normal

Figure 14. Forest Plot of Subgroup Analysis Results of the Primary Efficacy Endpoint



Source: Statistical reviewer, ADSL and SAS software were used.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CtNP, cycle-threshold nucleoprotein gene targets; rVSV, recombinant vesicular stomatitis virus; ULN, upper limit of normal

Table 72 was generated to verify (b) (4) because the age cutoff values were different from my previous age subgroup analyses.

Table 72. Twenty-Eight-Day Mortality Rates by Age Groups, ITT Concurrent, PALM Trail

Mortality Rate by Age Group	REGN-EB3 (N=154) Death/Total (%)	REGN-EB3 (N=154) (95% CI)[a]	ZMapp (N=153) Death/Total (%)	ZMapp (N=153) (95% CI)[a]
Overall	**52/154 (33.8%)**	(26.4%, 41.8%)	**78/153 (51.0%)**	(42.8%, 59.1%)
Adults (≥18 years)	**39/115 (33.9%)**	(25.4%, 43.3%)	**67/125 (53.6%)**	(44.5%, 62.6%)
<18 years	13/39 (33.3%)	(19.1%, 50.2%)	11/28 (39.3%)	(21.5%, 59.4%)
≥12 to <18 years of age	**2/8 (25.0%)**	(3.2%, 65.1%)	**4/8 (50.0%)**	(15.7%, 84.3%)
≥6 to <12 years of age	**1/8 (12.5%)**	(0.3%, 52.7%)	**1/4 (25.0%)**	(0.6%, 80.6%)
≥2 to <6 years of age	**4/10 (40.0%)**	(12.2%, 73.8%)	**3/6 (50.0%)**	(11.8%, 88.2%)
≥1 to <2 years of age	**3/8 (37.5%)**	(8.5%, 75.5%)	**3/7 (42.9%)**	(9.9%, 81.6%)
≥1 month to <1 year	**2/4 (50.0%)**	(6.8%, 93.2%)	**0/1 (0.0%)**	(0%, 97.5%)
<1 month	**1/1 (100%)**	(2.5%, 100%)	**0/2 (0.0%)**	(0%, 84.2%)

Source: Statistical reviewer; ADSL and SAS software used.
[a] Exact 95% confidence interval (CI)
Abbreviations: CI, confidence interval; ITT, intention-to-treat; N, number of subjects; PALM, PAmoja TuLinde Maisha

Sensitivity Subgroup Analyses by Stratification Factors in Different Analysis Populations

There were two randomization stratification factors—baseline viral load and study site (ETU):

- Baseline viral load: cycle-threshold nucleoprotein gene target (CtNP) ≤22 (high) vs. >22 (low).
- Site: Beni, Butembo, Katwa, and Mangina.

The results of subgroup analysis by baseline viral load were similar across analysis populations, comprising ITT concurrent, ITT overall, Treated, and ITT Amendment 3 (Table 73).

Table 73. Summary of 28-Day Mortality by CtNP Baseline Category Across Analysis Populations, PALM Trial

Population/ Subpopulation	REGN-EB3 Death/Total (%)	ZMapp Death/Total (%)	Rate Difference (95% CI)[a]	Boschloo's Two-Sided P-Value[b]
CtNP ≤22 at Baseline				
ITT Concurrent	42/66 (63.6%)	56/64 (87.5%)	-23.9 (-38.5, -7.3)	0.0015
ITT Overall	42/67 (62.7%)	60/70 (85.7%)	-23.0 (-37.8, -6.8)	0.0023
APT (Treated)	38/63 (60.3%)	55/63 (87.3%)	-27.0 (-41.8, -10.2)	0.0006
ITT Amd3	42/67 (62.7%)	56/64 (87.5%)	-24.8 (-39.1, -7.6)	0.0009
CtNP >22 at Baseline				
ITT Concurrent	10/88 (11.4%)	22/88 (25.0%)	-13.6 (-25.3, -1.9)	0.0215
ITT Overall	10/92 (10.9%)	24/98 (24.5%)	-13.6 (-24.6, -2.0)	0.0197
APT (Treated)	9/91 (9.9%)	23/89 (25.8%)	-16.0 (-27.4, -3.7)	0.0049
ITT Amd3	10/92 (10.9%)	23/89 (25.8%)	-15.0 (-26.5, -2.8)	0.0113

Source: Statistical reviewer; ADSL and SAS software used.
[a] The exact confidence interval was based on inverting two one-sided tests in StatXact.
[b]:P-value is based on Boschloo's test with a default gamma of 0 in StatXact.
Abbreviations: APT, all patients treated; CI, confidence interval; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha

The subgroup analysis results by site were similar across analysis populations, comprising ITT concurrent, ITT overall, Treated, and ITT Amendment 3 (Table 74).

Table 74. Summary of 28-Day Mortality by Site Across Analysis Populations, PALM Trial

Population/ Subpopulation	REGN-EB3 Death/Total (%)	ZMapp Death/Total (%)	Rate Difference (95% CI)[a]	Boschloo's Two-Sided P-Value[b]
Beni				
ITT Concurrent	23/67 (34.3%)	36/68 (52.9%)	-18.6 (-34.9, -1.3)	0.0324
ITT Overall	23/72 (31.9%)	42/84 (50.0%)	-18.1 (-33.1, -1.7)	0.0201
APT (Treated)	21/70 (30.0%)	37/69 (53.6%)	-23.6 (-39.2, -5.7)	0.0048
ITT Amd3	23/72 (31.9%)	37/69 (53.6%)	-21.7 (-37.3, -3.7)	0.0091
Butembo				
ITT Concurrent	21/63 (33.3%)	28/60 (46.7%)	-13.3 (-30.4, 4.4)	0.1293
ITT Overall	21/63 (33.3%)	28/60 (46.7%)	-13.3 (-30.4, 4.4)	0.1293
APT (Treated)	19/61 (31.2%)	28/60 (46.7%)	-15.5 (-32.5, 2.2)	0.0824
ITT Amd3	21/63 (33.3%)	28/60 (46.7%)	-13.3 (-30.4, 4.4)	0.1293
Katwa				
ITT Concurrent	4/10 (40.0%)	7/12 (58.3%)	-18.3 (-57.2, 25.3)	0.5876
ITT Overall	4/10 (40%)	7/12 (58.3%)	-18.3 (-57.2, 25.3)	0.5876
APT (Treated)	3/9 (33.3%)	6/11 (54.6%)	-21.2 (-61.0, 24.4)	0.3749
ITT Amd3	4/10 (40.0%)	7/12 (58.3%)	-18.3 (-57.2, 25.3)	0.5876
Mangina				
ITT Concurrent	4/14 (28.6%)	7/13 (53.9%)	-29.8 (-63.4, 12.1)	0.1767
ITT Overall	4/14 (28.6%)	7/13 (53.9%)	-29.8 (-63.4, 12.1)	0.1767
APT (Treated)	4/14 (28.6%)	7/13 (53.9%)	-29.8 (-63.4, 12.1)	0.1767
ITT Amd3	1/14 (28.6%)	7/13 (53.9%)	-29.8 (-63.4, 12.1)	0.1767

Source: Statistical reviewer; ADSL and SAS software used.
[a] The exact confidence interval was based on inverting two one-sided tests in StatXact.
[b] P-value is based on Boschloo's test with a default gamma of 0 in StatXact.
Abbreviations: APT, all patients treated; CI, confidence interval; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha

Sub-Subgroup Analysis for Viral Load at Baseline (CtNP Category) and the Number of days From Symptom Onset to Randomization and a Subset of Laboratory Measures

To gain insight into the relationships between baseline viral load and other baseline factors and their impact on the primary efficacy endpoint, the reviewer conducted the following sub-subgroup analyses.

Viral Load at Baseline and the Number of Days From Symptom Onset to Randomization

The 2×2 table of the subject counts by baseline viral load and the number of days from symptom onset to randomization is shown in Table 75. A chi-squared test of the association of the two factors had a two-sided P-value of 0.4059. There is no strong association between these two factors; i.e., low viral load at baseline is not strongly associated with early treatment (≤5 days from symptom onset to randomization).

Table 75. Subject Counts by Baseline CtNP value and the Number of days From Symptom Onset to Randomization, ITT Concurrent Population, PALM Trial

	Days From Symptom Onset to Randomization		
Baseline viral load	**≤5 Days**	**>5 Days**	**Number of Subjects**
CtNP >22 (row %)	76 (58%)	54 (42%)	130
CtNP ≤22 (row %)	112 (64%)	64 (36%)	176
Any baseline CtNP (row %)	188 (61%)	118 (39%)	306[1]

Source: Statistical reviewer; ADSL and SAS software used.
[1] One subject without CtNP measurement at baseline was missing from the table.
Abbreviations: CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha

The sub-subgroup analyses of the 28-day mortality rate are presented in Table 76. The viral load at baseline had the greatest effect on the 28-day mortality rate; the impact of the number of days

from symptom onset to randomization was small in the lower-baseline-viral-load group (CtNP >22) only.

Table 76. Sub-Subgroup Analyses of Viral Load and the Number of Days From Symptom Onset to Randomization on the Primary Efficacy Endpoint (28-Day Mortality), ITT Concurrent, PALM Trial

Population/ Subpopulation	REGN-EB3 (N=154) Death/Total (%)	ZMapp (N=153) Death/Total (%)	Rate Difference % (95% CI)[a]	Boschloo's Two-Sided P-value[b]
ITT Concurrent	52/154 (33.8%)	78/153 (51.0%)	-17.2 (-28.0, -4.1)	0.0023
Viral load across the number of days from symptoms onset to randomization				
CtNP ≤22, onset ≤5 days	28/43 (65.1%)	27/33 (81.8%)	-16.7 (-36.1, 4.1)	0.1016
CtNP ≤22, onset >5 days	14/23 (60.9%)	29/31 (93.6)	-32.7 (-55.2, -9.3)	0.0030
CtNP >22, onset ≤5 days	3/53 (5.7%)	13/59 (22.0%)	-16.4 (-30.2, -3.1)	0.0131
CtNP >22, onset >5 days	7/35 (20.0%)	9/29 (31.0%)	-11.0 (-33.1, 11.0)	0.3321

Source: Statistical reviewer; ADSL and SAS software used.
[a] The exact confidence interval was based on inverting two one-sided tests in StatXact.
[b] P-value is based on Boschloo's test with default gamma=0 in StatXact.
Abbreviations: CI, confidence interval; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; N, number of subjects; PALM, PAmoja TuLinde Maisha

Viral Load at Baseline and Baseline ALT, AST, and Creatinine

The 2×2 tables of the subject counts by baseline viral load and dichotomized baseline ALT, AST, and creatinine measures are listed in Table 77, Table 78, Table 79, Table 80, and Table 81. If baseline ALT, AST, and creatinine values are dichotomized, the P-values by chi-squared test of the association between baseline viral load and laboratory measures are <0.0001, indicating a strong association between the two factors; i.e., low viral load at baseline (CtNP >22) is strongly associated with less-abnormal baseline ALT (≤5 ULN), AST (≤5 ULN), and creatinine (≤3 mg/dL) value.

Table 77. Subject Counts by Baseline Viral Load and Baseline ALT Category (≤5 ULN vs. >5 ULN), ITT Concurrent, PALM Trial

VL\Baseline ALT	≤5 ULN	>5 ULN		Association Test:
CtNP >22	119 (83.2%)	24 (16.8%)	143	**Chi-squared Test –**
CtNP ≤22	17 (16.4%)	87 (83.7%)	104	**Two-Sided P-Value**
			247[1]	<0.0001

Source: Statistical reviewer; ADSL and SAS software used.
[1] Sixty subjects are missing from the table.
Abbreviations: ALT, alanine aminotransferase; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha; ULN, upper limit of normal; VL, viral load

Table 78. Subject Counts by Baseline Viral Load and Baseline ALT Category (≤10 ULN vs. >10 ULN), ITT Concurrent, PALM Trial

VL\Baseline ALT	≤10 ULN	>10 ULN		Association Test:
CtNP >22	132 (92.3%)	11 (7.7%)	143	**Chi-Squared Test –**
CtNP ≤22	50 (48.1%)	54 (51.9%)	104	**Two-Sided P-Value**
			247[1]	<0.0001

Source: Statistical reviewer; ADSL and SAS software used.
[1] Sixty subjects are missing from the table.
Abbreviations: ALT, alanine aminotransferase; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha; ULN, upper limit of normal; VL, viral load

Table 79. Subject Counts by Baseline Viral Load and Baseline AST Category (≤5 ULN vs. >5 ULN), ITT Concurrent, PALM Trial

VL\Baseline AST	≤5 ULN	>5 ULN		Association Test:
CtNP >22	75 (56.4%)	58 (43.6%)	133	Chi-Squared Test –
CtNP ≤22	1 (2.4%)	41 (97.6%)	42	Two-Sided P-Value
			175[1]	<0.0001

Source: Statistical reviewer; ADSL and SAS software used.
[1] One hundred thirty-two subjects are missing from the table.
Abbreviations: ALT, alanine aminotransferase; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha; ULN, upper limit of normal

Table 80. Subject Counts by Baseline Viral Load and Baseline AST Category (≤10 ULN vs. >10 ULN), ITT Concurrent, PALM Trial

VL\Baseline AST	≤10 ULN	>10 ULN		Association Test:
CtNP >22	90 (67.7%)	43 (32.3%)	133	Chi-Squared Test –
CtNP ≤22	5 (11.9%)	37 (88.1%)	42	Two-Sided P-Value
			175[1]	<0.0001

Source: Statistical reviewer; ADSL and SAS software used.
[*1] One hundred thirty-two subjects are missing from the table.
Abbreviations: AST, aspartate aminotransferase; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha; ULN, upper limit of normal; VL, viral load

Table 81. Subject Counts by Baseline Viral Load and Baseline Creatinine Category (≤3 mg/dL vs. >3 mg/dL), ITT Concurrent, PALM Trial

VL\Baseline Creatinine	≤3 mg/dL	>3 mg/dL		Association Test:
CtNP >22	120 (83.9%)	23 (16.1%)	143	Chi-Squared Test –
CtNP ≤22	55 (55.6%)	44 (44.4%)	99	Two-sided P-Value
			242[1]	<0.0001

Source: Statistical reviewer; ADSL and SAS software used.
[1] Sixty-five subjects are missing from the table.
Abbreviations: CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha; ULN, upper limit of normal; VL, viral load

Baseline CtNP and lab measures were treated as the continuous variables and line regression models were fitted between baseline CtNP and laboratory measures. The summary of linear regression models is listed in Table 82. It is consistent with the previous dichotomized analyses; i.e., it appears that there exists reasonable prediction relationship between baseline laboratory measures and the baseline viral load even though the R-square is relatively low for all three regression models, especially for baseline creatinine. The reviewer did not further assess the prediction model fitness. In summary, it appears that there is a correlation between low baseline viral load and less abnormal baseline lab measures (ALT, AST, and creatinine).

Table 82. Summary of Regression Analyses Between Baseline Viral Load and Baseline Laboratory Measures, ITT Concurrent, PALM Trial

Model	Model F-Test P-Value	R-Square	P-Value for Intercept	P-Value for Coefficient
VL=26.31 - 0.0072*ALT	<0.0001	0.3425	<0.0001	<0.0001
VL=28.63 - 0.0045*AST	<0.0001	0.4171	<0.0001	<0.0001
VL=24.66 - 0.3335*Creatinine	0.0054	0.0318	<0.0001	0.0054

Source: Statistical reviewer; ADSL and SAS software were used.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ITT, intention-to-treat; PALM, PAmoja TuLinde Maisha; VL, viral load

The sub-subgroup analysis results of the effect of baseline CtNP and laboratory measures on the 28-day mortality rate are presented in Table 83. Baseline viral load had the greatest effect on the 28-day mortality rate; the baseline laboratory measures exerted small add-on effects on the 28-day mortality rate. For example, the 28-day mortality rate for baseline creatinine >3 mg/dL was

higher than that for a baseline creatinine ≤3 mg/dL within baseline CtNP >22 and CtNP ≤22. However, the effect of baseline viral load was markedly greater than those of the three laboratory measures on the primary efficacy endpoint.

Table 83. Sub-Subgroup Analyses of Viral Load and Laboratory Measures on the Primary Efficacy Endpoint (28-Day Mortality), ITT Concurrent, PALM Trial

Population/Subpopulation	REGN-EB3 (N=154) Death/Total (%)	ZMapp (N=153) Death/Total (%)	Rate Difference % (95% CI)[a]	Boschloo's Two-Sided P-Value[b]
ITT Concurrent	52/154 (33.8%)	78/153 (51.0%)	-17.2 (-28.0, -4.1)	0.0023
Viral load cross ALT by 5× ULN				
CtNP ≤22, ALT ≤5× ULN	6/9 (66.7%)	5/8 (62.5%)	4.2 (-42.8, 49.7)	0.4921
CtNP ≤22, ALT >5× ULN	26/44 (59.1%)	38/43 (88.4%)	-29.3 (-46.7, -9.3)	0.0012
CtNP >22, ALT ≤5× ULN	4/65 (6.1%)	9/54 (16.7%)	-10.5 (-23.7, 1.1)	0.0441
CtNP >22, ALT >5× ULN	2/9 (22.2%)	9/15 (60.0%)	-37.8 (-70.1, 6.2)	0.0427
Viral load cross ALT by 10× ULN				
× ≤22, ALT ≤10× ULN	15/28 (53.6%)	16/22 (72.7%)	-19.2 (-44.6, 9.0)	0.0947
CtNP ≤22, ALT >10× ULN	17/25 (68.0%)	27/29 (93.1%)	-25.1 (-47.3, -3.8)	0.0118
CtNP >22, ALT ≤10× ULN	5/70 (7.1%)	12/62 (19.4%)	-12.2 (-24.9, -0.2)	0.0229
CtNP >22, ALT >10× ULN	1/4 (25.0%)	6/7 (85.7%)	-60.7 (-94.4, 9.2)	0.0351
Viral load cross AST by 5× ULN				
CtNP ≤22, AST ≤5× ULN	0/1 (0%)	0/0		
CtNP ≤22, AST >5× ULN	10/24 (41.7%)	13/17 (76.5%)	-34.8 (-60.5, -2.3)	0.0153
CtNP >22, AST ≤5× ULN	0/42 (0%)	7/33 (21.2%)	-21.2 (-38.9, -8.7)	0.0012
CtNP >22, AST >5× ULN	3/25 (12.0%)	10/33 (30.3%)	-18.3 (-39.7, 4.3)	0.0578
Viral load cross AST by 10× ULN				
CtNP ≤22, AST ≤10× ULN	3/5 (60.0%)	0/0		
CtNP ≤22, AST >10× ULN	7/20 (35.0%)	13/17 (76.5%)	-41.5 (-67.9, -7.4)	0.0063
CtNP >22, AST ≤10× ULN	1/48 (2.1%)	8/42 (19.1%)	-17.0 (-32.0, -4.2)	0.0050
CtNP >22, AST >10× ULN	2/19 (10.5%)	9/24 (37.5%)	-27.0 (-51.9, 1.0)	0.0244
Viral load cross Creatinine by 3 mg/dL				
CtNP ≤22, Creat ≤3 mg/dL	13/27 (48.2%)	22/28 (78.6%)	-30.4 (-54.0, -4.0)	0.0105
CtNP ≤22, Creat >3 mg/dL	17/24 (70.8%)	19/20 (95.0%)	-24.2 (-46.9, -0.5)	0.0302
CtNP >22, Creat ≤3 mg/dL	3/63 (4.8%)	9/57 (15.8%)	-11.0 (-24.0, 0)	0.0328
CtNP >22, Creat >3 mg/dL	3/11 (27.3%)	9/12 (75.0%)	-47.7 (-78.9, -2.9)	0.0178

Source: Statistical reviewer; ADSL and SAS software were used.
[a] The exact confidence interval was based on inverting two one-sided tests in StatXact.
[b] P-value is based on Boschloo's test with a default gamma of 0 in StatXact.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; Creat, creatinine; CtNP, cycle-threshold nucleoprotein gene targets; ITT, intention-to-treat; N, number of subjects; PALM, PAmoja TuLinde Maisha; ULN, upper limit of normal; VL, viral load

16.5. Twenty-Eight-Day Mortality Rates of ZMapp in PREVAIL II and PALM Trials

The overall 28-day mortality rate in the PALM trial was 51.0% (78/153) with Clopper-Pearson exact 95% CI [40.9%, 57.2%], while the 28-day mortality rate in the PREVAIL II trial was 22.2% (8/36) with a Clopper-Pearson exact 95% CI of 10.1% to 39.2%. Examination of the proportions of baseline viral load categories, which are almost identical (Table 84 and Table 85), shows that the difference in mortality rates between the PREVAIL II and PALM trials was due to factors other than baseline viral load. The 28-day mortality rates of the low and high baseline viral load groups were higher for ZMapp in the PALM trial compared to the PREVAIL II trial.

The study protocol stated that "a mortality rate of 30% in the ZMapp + oSOC control arm was based, in part, on a meta-analysis of eight clinical studies conducted during the 2014 to 2016 West African Ebola outbreak. This analysis indicated that mortality rates within PREVAIL II were lower than other studies across both treatment and control arms. Hence, the expected mortality rate with ZMapp in this trial may be higher than the point estimate from PREVAIL II."

Table 84. Subgroup Analysis of Primary Endpoint by Baseline Viral Load in the PREVAIL II Trial

	Proportion (N=71)		28-Day Mortality Rate	
	ZMapp (n=36)	oSOC (n=35)	ZMapp	oSOC
CtNP ≤22	15 (42%)	15 (43%)	7/15 (46.7%)	9/15 (60%)
CtNP >22	21 (58%)	20 (57%)	1/21 (4.8%)	4/20 (20%)

Source: Dr. Daniel Rubin's Statistical Review for IND 125530/SN0043 on July 15, 2016 for PREVAIL II Trial
Abbreviations: CtNP, cycle-threshold nucleoprotein gene targets; N, number of subjects; n, number of subjects in subgroup; oSOC, optimized standard of care

Table 85. Subgroup Analysis of Primary Endpoint by Baseline Viral Load in the PALM Trial

	Proportion (N=307)		28-Day Mortality Rate	
	ZMapp (n=152[1])	REGN-EN3 (n=154)	ZMapp	REGN-EN3
CtNP ≤22	64 (42%)	66 (43%)	56/64 (87.5%)	42/66 (63.6%)
CtNP >22	88 (58%)	88 (57%)	22/88 (25.0%)	10/88 (11.4%)

Source: Statistical reviewer, ADSL and SAS software were used.
[1] One subject did not have a Ct value because he/she was 1 day of age.
Abbreviations: CtNP, cycle-threshold nucleoprotein gene targets; N, number of subjects; n, number of subjects in subgroup; PALM, PAmoja TuLinde Maisha

17. Clinical Safety Assessment Additional Information and Assessment

The following table (Table 86) (b) (4)

Table 86. Adverse Events Occurring at ≥1% in the REGN-EB3 Arm, Safety Population, PALM Trial

Adverse Event [a]	REGN-EB3 (N=154) n (%)	ZMapp [c] (N=168) n (%)
Any adverse event	120 (77.9)	149 (88.7)
Pyrexia[b]	83 (53.9)	97 (57.7)
Chills[b]	60 (39.0)	55 (32.7)
Tachycardia[b]	31 (20.1)	53 (31.5)
Tachypnea[b]	30 (19.5)	47 (28.0)
Vomiting	30 (19.5)	38 (22.6)
Hypotension[b]	23 (14.9)	52 (31.0)
Diarrhea[b]	17 (11.0)	31 (18.5)
Hypoxia	16 (10.4)	19 (11.3)
Hypertension[b]	12 (7.8)	17 (10.1)
Nausea	8 (5.2)	12 (7.1)
Dyspnea[b]	7 (4.5)	12 (7.1)
Headache	6 (3.9)	8 (4.8)
Cough	5 (3.2)	6 (3.6)
Anorexia	5 (3.2)	6 (3.6)
Asthenia	5 (3.2)	4 (2.4)
Agitation	4 (2.6)	8 (4.8)
Abdominal pain upper	4 (2.6)	3 (1.8)
Pruritus[b]	3 (1.9)	2 (1.2)
Abdominal pain	3 (1.9)	5 (3.0)
Chest pain[b]	2 (1.3)	7 (4.2)
Tremor	2 (1.3)	0
Seizure	2 (1.3)	6 (3.6)
Vertigo	2 (1.3)	5 (3.0)

Source: Reviewer's analysis of adfa2.xpt dataset using JReview. Filter applied to "parcat3": "Adverse Reactions during Infusion coded term", "Other Adverse Reactions during Infusion coded term." List of terms are in descending order of incidence in the REGN-EB3 arm.
[a] Adverse events in this table were reported on the day of infusion, and included signs and symptoms that occurred during or immediately after infusion. These terms were reported in the CRF, but terms from narratives for subject death reports were not included. MedDRA (Version 22.0) coding dictionary applied.
[b] Adverse events that were prespecified
[c] Adverse events were reported on the day of infusion; ZMapp was to be administered as three separate infusions on up to three separate days.
Abbreviations: N, number of subjects; n, number of subjects in subgroup; PALM, PAmoja TuLinde Maisha

Table 87 includes events occurring in at least 10% of pediatric subjects in the REGN-EB3 arm. (b) (4) the overall profile of events was similar to that in adult subjects.

Table 87. Summary of Adverse Reactions During or After Infusion Reported by ≥10% of Subjects in the REGN-EB3 Arm, Safety Population, Subjects Under 18 Years of Age, PALM Trial

Adverse Event[a]	REGN-EB3 (N=39) n (%)	ZMapp [c] (N=32) n (%)
Any adverse event	30 (76.9)	28 (87.5)
Pyrexia[b]	17 (43.6)	21 (65.6)
Tachycardia[b]	9 (23.1)	8 (25.0)
Chills[b]	9 (23.1)	7 (21.9)
Vomiting	8 (20.5)	7 (21.9)
Tachypnea[b]	7 (17.9)	8 (25.0)
Hypotension[b]	6 (15.4)	7 (21.9)
Diarrhea[b]	4 (10.3)	6 (18.8)
Dyspnea[b]	4 (10.3)	1 (3.1)
Nausea	4 (10.3)	1 (3.1)

Source: Reviewer's analysis of adfa2.xpt dataset using JReview. Filter applied to "parcat3": "Adverse Reactions during Infusion coded term", "Other Adverse Reactions during Infusion coded term", "AE Term from Death Narratives coded term." List of terms are in descending order of incidence in the REGN-EB3 arm.
[a] Adverse events in this table were reported on the day of infusion, and included signs and symptoms that occurred during or immediately after infusion. These terms were reported in the CRF and extracted from narratives for subject death reports. MedDRA (Version 22.0) coding dictionary applied.
[b] Adverse events that were prespecified.
[c] Adverse events were reported on the day of infusion; ZMapp was to be administered as three separate infusions on up to three separate days.
Abbreviations: N, number of subjects; n, number of subjects with adverse event; PALM, PAmoja TuLinde Maisha

Table 88. Subjects Meeting Laboratory Abnormality Criteria, Cumulative Worsened Grade From Baseline, PALM Trial, Safety Population, Subjects Under 18 Years of Age, PALM Trial

Laboratory Test	REGN-EB3 N=39 n (%)	ZMapp N=32 n (%)
Sodium, increased		
Any grade (≥146 mmol/L)	11 (28.2)	6 (18.8)
Grade 3 or 4 (≥154 mmol/L)	5 (12.8)	3 (9.4)
Sodium, decreased		
Any grade (<135 mmol/L)	21 (53.8)	7 (21.9)
Grade 3 or 4 (<125 mmol/L)	2 (5.1)	4 (12.5)
Potassium, increased		
Any grade (≥5.6 mmol/L)	17 (43.6)	10 (31.2)
Grade 3 or 4 (≥6.5 mmol/L)	11 (28.2)	7 (21.9)
Potassium, decreased		
Any grade (<3.4 mmol/L)	9 (23.1)	5 (15.6)
Grade 3 or 4 (<2.5 mmol/L)	4 (10.3)	3 (9.4)
Creatinine, increased		
Any grade (≥1.1× ULN [a])	14 (35.9)	11 (34.4)
Grade 3 or 4 (>1.8× ULN [a] or increase to ≥1.5× baseline)	11 (28.2)	11 (34.4)
Alanine aminotransferase (U/L)		
Any grade (≥1.25× ULN)	10 (25.6)	7 (21.9)
Grade 3 or 4 (≥5× ULN)	3 (7.7)	5 (15.6)
Aspartate aminotransferase (U/L)		
Any grade (≥1.25× ULN)	9 (23.1)	3 (9.4)
Grade 3 or 4 (≥5× ULN)	6 (15.4)	1 (3.1)

Source: ad b.xpt, Software: R
Grading scale used was DAIDS corrected version 2.1.
[a] ULN for serum creatinine=1.2 mg/dL. An increase to ≥1.5 x from baseline was applied if the worsening grade was higher.
Abbreviations: N, number of subjects with relevant laboratory data; n, number of subjects with abnormality; PALM, PAmoja TuLinde Maisha; ULN, upper limit of normal

Analyses by race were not conducted because race information was not collected. Analyses of adverse events occurring during or post infusion by sex and age group are presented in **Table 88** and **Table 89**.

Table 89. Subgroup Analysis by Gender for AEs Occuring During or Post-Infusion, Safety Population, Trial RCT-19-I-0003

	REGN-EB3 N=154		ZMapp N=168	
Adverse Event	**Male N=63 n (%)**	**Female N=91 n (%)**	**Male N=82 n (%)**	**Female N=86 n (%)**
Any adverse event	49 (77.8)	71 (78)	74 (90.2)	75 (87.2)
Chills	24 (38.1)	35 (38.5)	33 (40.2)	22 (25.6)
Diarrhea	9 (14.3)	8 (8.8)	18 (22)	13 (15.1)
Dyspnea	5 (7.9)	2 (2.2)	5 (6.1)	7 (8.1)
Hypotension	8 (12.7)	15 (16.5)	21 (25.6)	31 (36)
Nausea	2 (3.2)	6 (6.6)	7 (8.5)	5 (5.8)
Pyrexia	32 (50.8)	51 (56)	53 (64.6)	44 (51.2)
Tachycardia	14 (22.2)	17 (18.7)	22 (26.8)	31 (36)
Tachypnoea	13 (20.6)	17 (18.7)	23 (28)	23 (26.7)
Vomiting	9 (14.3)	21 (23.1)	21 (25.6)	17 (19.8)

Source: adfa2.xpt; Software: R
Abbreviations: N, number of subjects; n, number of subjects with adverse event; REGN-EB3, atoltivimab-odesivimab-maftivimab-ebgn
Filter applied to "parcat3": "Adverse Reactions during Infusion coded term" and "Other Adverse Reactions during Infusion coded term".

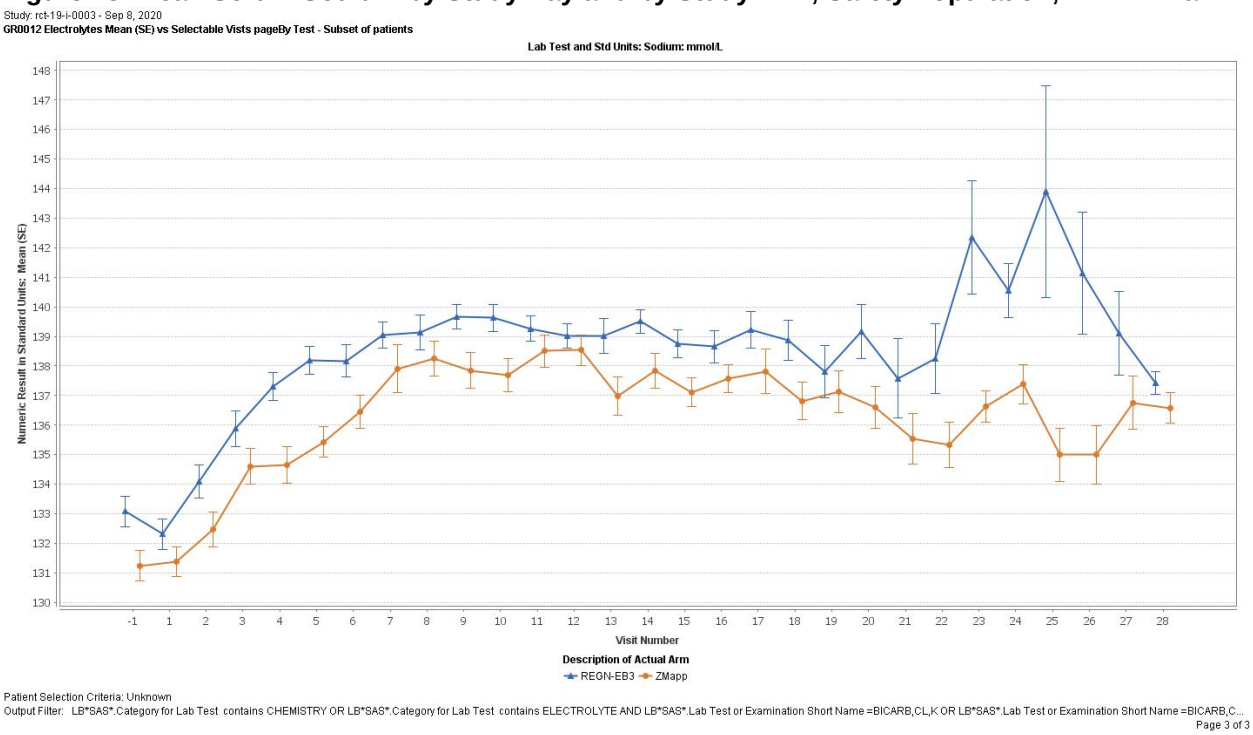
Table 90. Subgroup Analysis by Age Groups for AEs Occuring During or Post-Infusion, Safety Population, Trial RCT-19-I-0003

	REGN-EB3 N=154				ZMapp N=168			
Adverse Event	**Age <6 N=24 n (%)**	**Age 6-12 N=7 n (%)**	**Age 12-18 N=8 n (%)**	**Age ≥18 N=115 n (%)**	**Age <6 N=19 n (%)**	**Age 6-12 N=5 n (%)**	**Age 12-18 N=8 n (%)**	**Age ≥18 N=136 n (%)**
Any adverse event	19 (79.2)	6 (85.7)	5 (62.5)	90 (78.3)	16 (84.2)	4 (80)	8 (100)	121 (89)
Chills	5 (20.8)	3 (42.9)	0	51 (44.3)	2 (10.5)	1 (20)	4 (50)	48 (35.3)
Diarrhea	1 (4.2)	1 (14.3)	1 (12.5)	14 (12.2)	3 (15.8)	2 (40)	1 (12.5)	25 (18.4)
Dyspnea	2 (8.3)	0	1 (12.5)	4 (3.5)	1 (5.3)	0	0	11 (8.1)
Hypotension	3 (12.5)	1 (14.3)	2 (25)	17 (14.8)	3 (15.8)	1 (20)	3 (37.5)	45 (33.1)
Nausea	1 (4.2)	1 (14.3)	2 (25)	4 (3.5)	0	0	1 (12.5)	11 (8.1)
Pyrexia	13 (54.2)	3 (42.9)	1 (12.5)	66 (57.4)	14 (73.7)	2 (40)	5 (62.5)	76 (55.9)
Tachycardia	9 (37.5)	0	0	22 (19.1)	4 (21.1)	1 (20)	3 (37.5)	45 (33.1)
Tachypnea	5 (20.8)	2 (28.6)	0	23 (20)	5 (26.3)	1 (20)	2 (25)	38 (27.9)
Vomiting	5 (20.8)	1 (14.3)	2 (25)	22 (19.1)	4 (21.1)	0	3 (37.5)	31 (22.8)

Source adfa2.xpt; Software: R
Abbreviations: N, number of subjects; n, number of subjects with adverse event; REGN-EB3, atoltivimab-odesivimab-maftivimab-ebgn
Filter applied to "parcat3": "Adverse Reactions during Infusion coded term" and "Other Adverse Reactions during Infusion coded term".

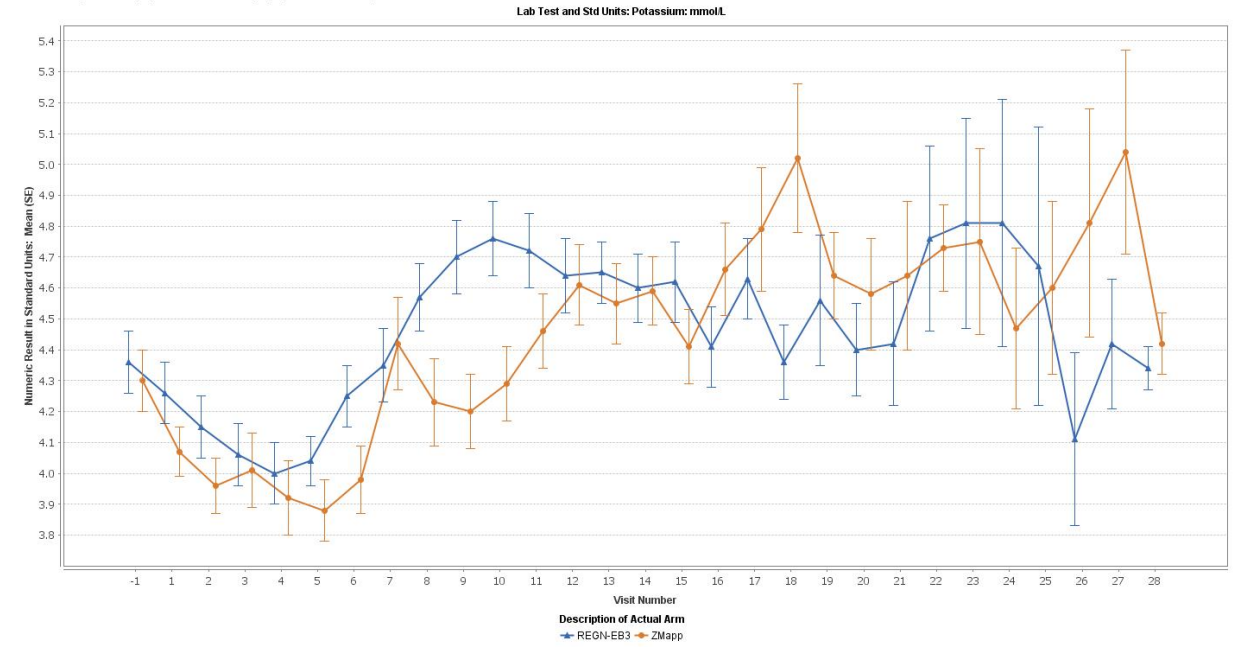
Figure 15, Figure 16, Figure 17, Figure 18, and Figure 19 show the mean daily laboratory values for the REGN-EB3 and ZMapp arms from baseline to Day 28 of the PALM trial. Chemistry values (AST, ALT, AST/ALT ratio, creatinine) by study day were the prespecified secondary efficacy endpoints. Consistent with the trend of death distribution by study day (where 68% of deaths occurred by Day 3), most of the laboratory values normalized by Day 7, driven by the treatment effect (or lack thereof) of the study drugs. This normalization of laboratory values reflected recovery of renal and hepatic function in survivors. Differences between the treatment arms, however, did not reveal trends suggestive of any safety concern.

Figure 15. Mean Serum Sodium by Study Day and by Study Arm, Safety Population, PALM Trial



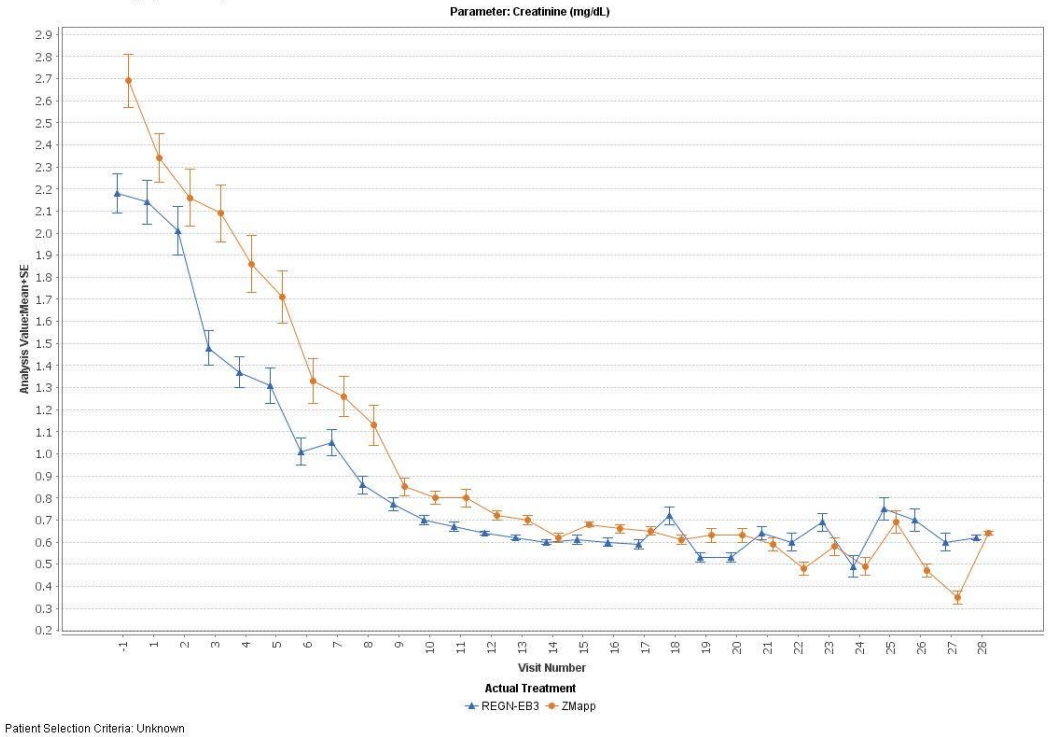
Source: Reviewer's analysis of the adlb.xpt dataset using JReview.
Abbreviations: PALM, PAmoja TuLinde Maisha

Figure 16. Mean Serum Potassium by Study Day and by Study Arm, Safety Population, PALM Trial



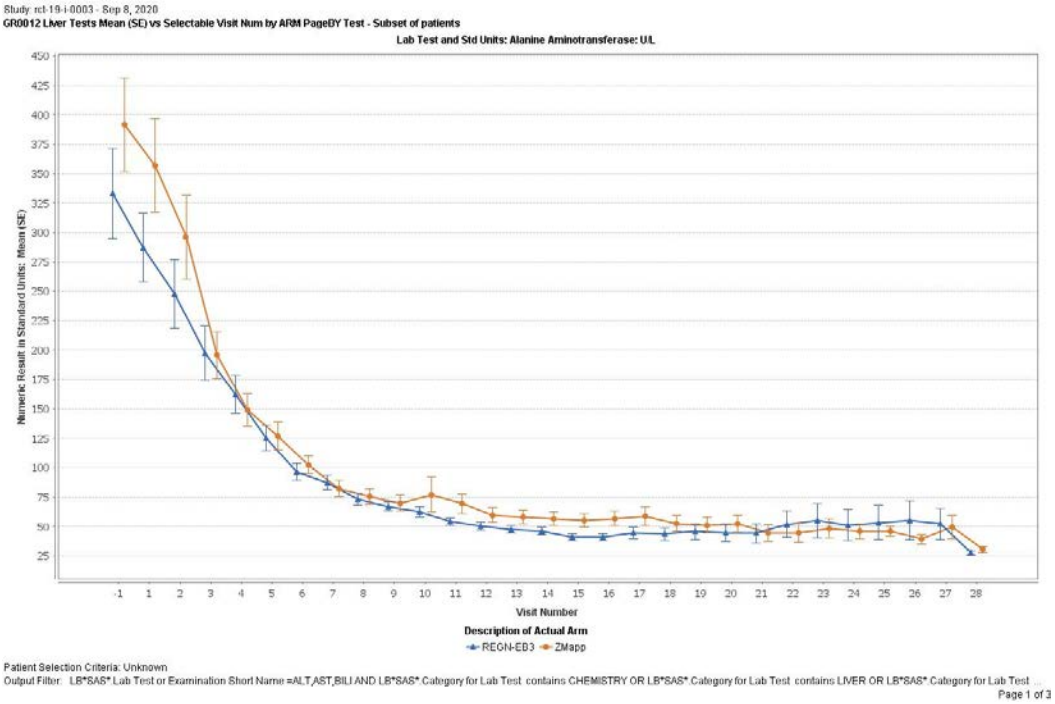
Source: Reviewer's analysis of the adlb.xpt dataset using JReview.
Abbreviations: PALM, PAmoja TuLinde Maisha

Figure 17. Mean Serum Creatinine by Study Day and by Study Arm, Safety Population, PALM Trial



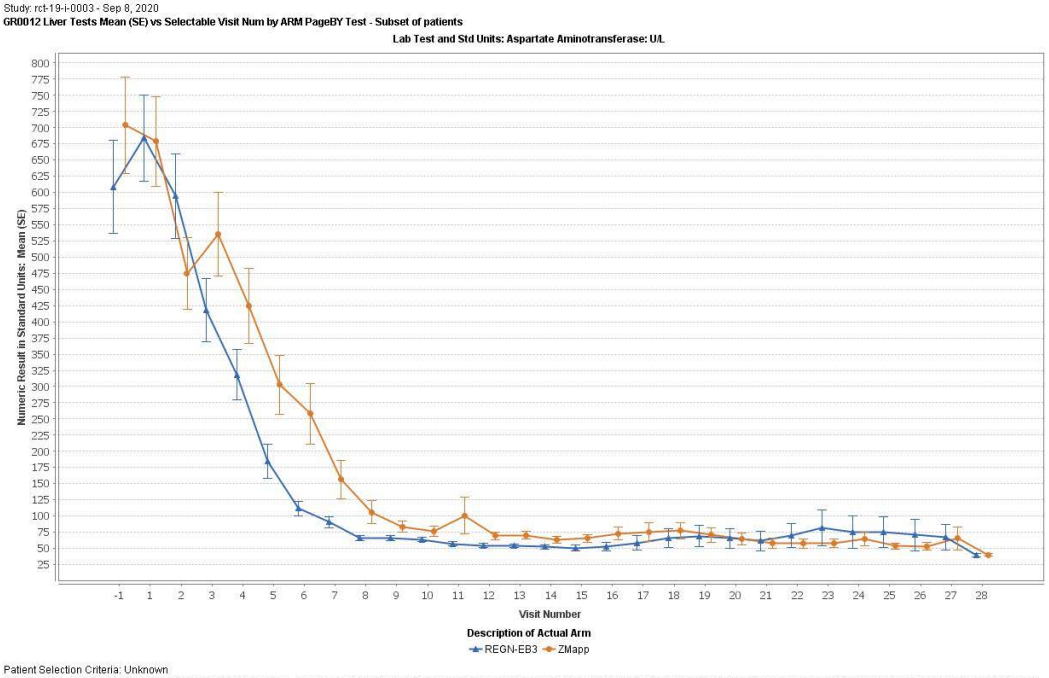
Source: Reviewer's analysis of the adlb.xpt dataset using JReview.
Abbreviations: PALM, PAmoja TuLinde Maisha

Figure 18. Mean Alanine Aminotransferase by Study Day and by Study Arm, Safety Population, PALM Trial



Source: Reviewer's analysis of the adlb.xpt dataset using JReview.
Abbreviations: PALM, PAmoja TuLinde Maisha

Figure 19. Mean Aspartate Aminotransferase by Study Day and by Study Arm, Safety Population, PALM Trial



Source: Reviewer's analysis of the adlb.xpt dataset using JReview.
Abbreviations: PALM, PAmoja TuLinde Maisha

18. Mechanism of Action/Drug Resistance Additional Information and Assessment

18.1. Virology: Additional Information and Assessment

18.1.1. Introduction and Background

The virus family *Filoviridae* is of the order *Mononegavirales* and comprises three genera: *Cuevavirus, Ebolavirus,* and *Marburgvirus*. To date, five species of *Ebolavirus* have been identified—Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV), Reston (RESTV), Bombali (BOMV) and Tai Forest (TAFV). A 2014 study found that five of six mAb epitopes for ZMapp™ and REGN-EB3 were conserved among *Zaire ebolavirus* outbreak strains but much less so for other *Ebolavirus* species (Ponomarenko et al. 2014) and therefore REGN-EB3 is not expected to be efficacious against other *Ebolavirus* species. Filoviruses are negative-strand RNA viruses that cause severe hemorrhagic disease characterized by high mortality rates. The virus initially infects monocytes, macrophages, and dendritic cells and spreads systemically to produce a primary viremia that leads to infection of other cell types including vascular endothelial cells. Virus replication leads to a rise in inflammatory cytokine levels and development of coagulopathies resulting in vascular leakage, hypovolemic shock, and multiorgan failure. The mean overall case fatality rate for all known EBOV cases as of this writing is 43.92±0.7% with the two most recent outbreaks recording case fatality rates of 66.0% for the 2018 to 2020 outbreak in the DRC (3,481 total cases) and 39.5% for the 2013 to 2016 Western Africa outbreak (28,652 total cases).

EBOV has a linear, single-stranded, negative-sense RNA genome that is ~19 kb in length and encodes seven structural proteins and several nonstructural proteins from seven genes (Jacob et al. 2020). Of these EBOV proteins and genes, two are discussed in this review, including the nucleoprotein (NP) gene, which is one of the targets of the reverse transcription-polymerase chain reaction (RT-PCR) assays used to assess EBOV infection. The GP gene is also detected in the RT-PCR assay and its product is the target of the REGN-EB3 cocktail. EBOV cell tropism is primarily determined by the EBOV GP, which interacts with attachment factors on the host cell surface and binds with the Niemann–Pick C1 protein receptor in the lysosome of infected host cells to initiate fusion and release of the viral genome into the cytoplasm (Jacob et al. 2020).

The EBOV envelope GP constitutes a promising target for antibody-based therapeutics against EBOV because it mediates both viral attachment and fusion with host cells. Blocking GP function with antibodies that bind to the EBOV GP has shown postexposure protection of EBOV-infected nonhuman primates (NHP) with ZMapp™ when treatment is initiated on Day 5 with subsequent doses on days 8 and 11 after challenge (Qiu et al. 2014). ZMapp™ is a mAb cocktail being developed by LeafBio, Inc. that was used as a control in the PALM trial. It is composed of three recombinant mouse/human chimeric IgG1κ mAbs—c13C6, c2G4, and c4G7; each was derived from three mouse mAbs directed against the EBOV Mayinga variant GP. REGN-EB3 also consists of three recombinant human mAbs derived using an EBOV Makona 2014 variant. A recent study found that five of six mAb epitopes for ZMapp™ and REGN-EB3 were conserved amongst EBOV outbreak strains but much less so for other *Ebolavirus* species

(Ponomarenko et al. 2014); therefore, REGN-EB3 is not expected to be efficacious against these. Maftivimab (REGN3479) and atoltivimab (REGN3470) bind noncompetitively to full-length GP but not secreted GP (sGP) and are neutralizing (maftivimab (REGN3479) is a potent neutralizer of GP function, but atoltivimab (REGN3470) neutralizes GP function in virus-like particle (VLP)-based assays but not those that use authentic EBOV). Odesivimab (REGN3471) binds to sGP and does not neutralize virus, but reportedly has antibody-dependent cellular cytotoxicity (ADCC) activity. REGN-EB3 has also exhibited postexposure protection in NHPs infected with EBOV-Kikwit when administered intravenously on days 5, 8, and 11.

Several studies have indicated that, in general, cocktails of mAbs targeting different EBOV GP epitopes have much greater antiviral activity in the NHP model than individual mAbs used as monotherapy (Table 91) (Olinger et al. 2012; Qiu et al. 2012; Pettitt et al. 2013; Qiu et al. 2013; Qiu et al. 2014).

Table 91. Efficacy of Individual and Combined Monoclonal Antibody Treatments in Guinea Pigs and Nonhuman Primates

Treatment groups, time of treatment	Dose (mg)	Mean time to death (days ± s.d.)	No. survivors/total	Survival (%)	Weight loss (%)	*P* value, compared with	
						cZMAb	MB-003
Guinea pigs						–	–
PBS, 3 dpi	N/A	7.3 ± 0.5	0/4	0	9%	–	–
cZMAb, 3 dpi	5	11.6 ± 1.8	1/6	17	7%	–	–
MB-003, 3 dpi	5	8.2 ± 1.5	0/6	0	40%	–	–
ZMapp1, 3 dpi	5	9.0 ± 0.0	4/6	67	<5%	0.190	0.0147
ZMapp2, 3 dpi	5	8.3 ± 0.6	3/6	50	8%	0.634	0.0692
ZMapp3, 3 dpi	5	8.6 ± 1.1	1/6	17	9%	0.224	0.411
c13C6, 1 dpi	5	8.4 ± 1.7	1/6	17	9%	–	–
h13F6, 1 dpi	5	10.2 ± 1.8	1/6	17	21%	–	–
c6D8, 1 dpi	5	10.5 ± 2.2	0/6	0	38%	–	–
Nonhuman primates							
PBS, 1 dpi	N/A	8.4 ± 1.9	0/1	0			
MB-003, 1 dpi	50	14.0 ± 2.8	1/3	33			
c13C6, 1 dpi	50	9.0 ± 1.4	1/3	33			
h13F6, 1 dpi	50	9.0 ± 2.0	0/3	0			
c6D8, 1 dpi	50	9.7 ± 0.6	0/3	0			

Source: Table 1, copied from Qiu et al. 2014)
Abbreviations: DPI, days post infection; N/A, not application

A cocktail comprising two or more mAbs is thought to confer protection via complementary neutralization and neutralization-independent mechanisms, and may reduce the opportunity for selection of escape mutants (Murin et al. 2014). In addition, the positioning of the epitope on the GP structure will likely determine if a particular mAb is neutralizing. Antibodies against the mucin-like domains of the EBOV GP are generally non-neutralizing because these domains, as well as any antibodies bound to them, are stripped from the viral surface by host cathepsins in the endosome, leaving behind an antibody-free, functional receptor-binding core of GP (Murin et al. 2014).

An important consideration for mAb cocktails is whether or not one or more of the mAbs bind to sGP. sGP is the soluble, dimeric version of GP that results from the primary open reading frame of the GP gene and is expressed abundantly during EBOV infection (Sanchez et al. 1998). An insertion/deletion in the EBOV GP gene sequence arises in the viral population after passage in cell culture, resulting in an insertion of a uridine at the poly-U site at positions 6918 to 6924, shifting it from a 7U to an 8U genotype. This change occurs within 24 hours postinfection in cell culture and flips the normal production ratios of sGP:GP such that GP is the dominant product with the 8U genotype (Volchkov et al. 1995; Kugelman et al. 2012). Importantly, mAbs that bind sGP may not be effective in protecting against infection, because sGP could serve as a decoy for

mAbs that might otherwise bind viral particles (Murin et al. 2014). Of note, one of the REGN-EB3 mAbs, odesivimab (REGN3471) binds to sGP.

18.1.1.1. Methodology

Reverse Transcriptase-Polymerase Chain Reaction

The Cepheid GeneXpert RT-PCR assay was used to document positive EBOV infection for enrollment into the PALM clinical trial and to assess EBOV viral load at baseline and at various timepoints in clinical trial 19-I-0003 (PALM RCT). The Cepheid GeneXpert assay is a real-time two-target RT-PCR assay for the qualitative detection of EBOV RNA. The assay separately quantifies the GP and NP genes of EBOV with results reported as cycle threshold (Ct) values, with an upper Ct level of 45 for both genes. Viral load (i.e., the number of copies of RNA/mL) and Ct are inversely correlated because greater concentrations of virus are detected in fewer cycles. Thus, a high Ct value denotes a low viral load and a low Ct value a high viral load (CtNP ≤22, high viral load and CtNP >22, low viral load). For reference, the Applicant stated that a CtNP <22 is equivalent to ≥7 $\log_{10}$ RNA copies/mL and a CtNP >22 is equivalent to <7 $\log_{10}$ RNA copies/mL. The RT-PCR assay for EBOV reports both CtNP and CtGP; however, because the assay is more sensitive for the NP target, CtNP was used in all analyses. (b) (5)

Conservation of REGN-EB3–Binding Sites

A bioinformatics investigation was performed to assess the genetic diversity of the EBOV GP regions protected by the individual REGN-EB3 mAbs in the hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) study using 2,543 publicly available EBOV genome sequences submitted to the National Center for Biotechnology Information database from 1976 to 2018. The most frequent polymorphisms observed in the atoltivimab (REGN3470)- and odesivimab (REGN3471)-protected regions occurred at a frequency of 0.20% (GP_L239S) and 0.34% (GP_D150A), respectively. The most frequent polymorphism in the maftivimab (REGN3479)-protected region, the T544I substitution, had a frequency of 2.0%. However, because this substitution commonly arises during virus propagation in a laboratory setting, it is unclear whether it is associated with circulating EBOV genomes or is the product of tissue culture passage (Hoffmann et al. 2017; Ruedas et al. 2017; Ueda et al. 2017). A single-nucleotide polymorphism (SNP) yielding the E280G substitution was identified in late 2019 during the North Kivu EBOV outbreak. The E280 residue is located within the atoltivimab (REGN3470) mAb footprint on EBOV GP identified in previous studies using HDX-MS (Section III.18.1.2.1).

Phenotypic Analysis of Substitutions

Phenotypic analyses of select EBOV substitutions were performed using EBOV Makona GP VLPs generated by cotransfecting HEK293T cells with a mix of plasmid constructs expressing EBOV Makona GP (wild-type or variant of interest), human immunodeficiency virus (HIV) Gag-Pol, and an HIV proviral vector encoding firefly luciferase.

18.1.1.2. Prior FDA Virology Reviews

A total of 27 Clinical Virology reviews was written for REGN-EB3 during development. Four submissions were reviewed as pre-IND submissions from March 31, 2015 to October 30, 2015.

A total of 23 submissions was reviewed during the IND development phase from December 21, 2015 to March 27, 2020.

18.1.1.3. State of Antivirals Used for the Indication Sought

There are currently no drugs approved for the treatment of EBOV infection.

18.1.2. Nonclinical Virology

18.1.2.1. Mechanism of Action

The Applicant provided two nonclinical virology study reports describing experiments performed to assess the mechanisms of action of the three mAbs of the REGN-EB3 cocktail.

Study Title: Determination of the Binding Properties of REGN3470, REGN3471, and REGN3479 to Ebola Virus Spike Glycoprotein

Study Number: REGN3479-MX-15041-sr-01v1

Objectives: The objectives of this study were:

- To determine the kinetic binding parameters by surface plasmon resonance (SPR) Biacore analysis for the interaction of individual mAbs atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) with EBOV Makona GP (recombinant GP.10xhis protein). These binding studies were performed at pH 7.4 and at 25°C.
- To determine the pH sensitivity of the dissociation rate constant of atoltivimab (REGN3470), REGN3741, and REGN3749 following binding to EBOV GP.10xhis at pH 7.4 and at 37°C by SPR Biacore analysis.
- To assess if atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) bind to recombinant EBOV Makona sGP protein (sGP.mmh) using the Octet HTX interferometer.
- To assess binding of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) to EBOV GP.10xhis under reducing and denaturing conditions by western blot analysis.
- To determine if REGN3740, REGN3741, and REGN3749 compete for binding to recombinant EBOV Makona GP.10xhis protein using antibody-paired Octet HTX assays.
- To determine whether the three mAbs in the REGN-EB3 mAb cocktail can bind simultaneously to EBOV Makona GP.10xhis protein following sequential addition by SPR Biacore analysis.
- To determine the potency of the REGN-EB3 mAb cocktail and the individual mAbs atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) to block EBOV Makona GP.10xhis in solution from binding to plate-coated atoltivimab (REGN3470), odesivimab (REGN3471), or maftivimab (REGN3479) by sandwich competition enzyme-linked immunosorbent assays.

Results

Kinetic binding parameters. The Applicant used SPR biosensor technology to measure the kinetic binding parameters for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) at 25°C and pH 7.4. The results indicated high-affinity binding of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) to

recombinant EBOV GP (EBOV Zaire variant Makona 2014). The equilibrium dissociation constant (K_D) values determined for two different lots of each of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) were 7.74 and 7.93nM, 8.42 and 8.10nM, and 2.97 and 3.70nM, respectively (Table 92).

Table 92. Summary of Kinetic Binding Parameters for the Interaction of Atoltivimab (REGN3470), Odesivimab (REGN3471), and Maftivimab (REGN3479) With Recombinant EBOV GP.10xhis at 25°C

		EBOV GP.10xhis Kinetic Binding Parameters			
Antibody	**Lot #**	**k_a ($M^{-1}s^{-1}$)**	**k_d (s^{-1})**	**K_D (M)**	**$T_{1/2}$ (Min)**
REGN3470	REGN3470-L5	2.80×10^{4}	2.22×10^{-4}	7.93×10^{-9}	52.0
REGN3470	9018800002.PCS	3.05×10^{4}	2.36×10^{-4}	7.74×10^{-9}	48.9
REGN3471	REGN3471-L5	1.69×10^{4}	1.37×10^{-4}	8.11×10^{-9}	84.3
REGN3471	9019300002.PCS	1.45×10^{4}	1.22×10^{-4}	8.41×10^{-9}	94.7
REGN3479	REGN3479-L5	2.16×10^{4}	8.00×10^{-5}	3.70×10^{-9}	144.4
REGN3479	9019800002.PCS	4.81×10^{4}	1.43×10^{-4}	2.97×10^{-9}	80.8

Source: Table 3, page 19, Study Report REGN3479-MX-15041-sr-01v1).
Abbreviations: EBOV, *Zaire ebolavirus;* GP, glycoprotein; k_a, association rate constant; k_d, dissociation rate constant; K_D, equil brium dissociation constant; T½, dissociative half-life; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Binding kinetics under acidic conditions. The Applicant noted that infection of EBOV is presumed to occur in the endosomal compartment where the pH is lower than normal physiologic pH, and ranges from approximately pH 6 to pH 5 (Lee and Saphire 2009). Therefore, they performed experiments to determine if the anti-EBOV GP mAbs retain binding to EBOV Makona GP.10xhis at these pH conditions. The assay format utilized binding of mAb to a GP.10xhis biosensor surface at pH 7.4, followed by mAb dissociation at pH 5.0, 6.0, or 7.4 at 37°C. Atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) displayed a less than 2- to 3-fold decline in dissociative $t_{1/2}$ values, indicating that acidic conditions (pH 5) should not have a major effect on the binding kinetics (Table 93).

Table 93. Dissociation Rate Constant and Dissociative Half-Life of Atoltivimab (REGN3470), Odesivimab (REGN3471), or Maftivimab (REGN3479) From EBOV GP at pH 5.0, 6.0 and 7.4

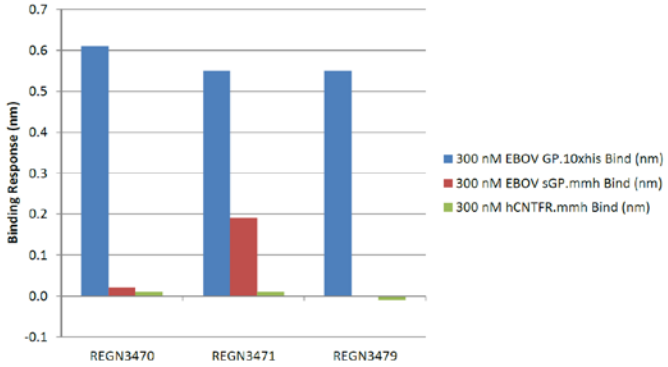
	pH 7.4		pH 6.0		pH 5.0	
Antibody	**k_d (1/s)**	**$t_{1/2}$ (Min)**	**k_d (1/s)**	**$t_{1/2}$ (Min)**	**k_d (1/s)**	**$t_{1/2}$ (Min)**
REGN3470	4.32×10^{-4}	26.7	3.67×10^{-4}	31.4	4.91×10^{-4}	23.5
REGN3471	1.95×10^{-4}	59.1	2.21×10^{-4}	52.3	3.32×10^{-4}	34.8
REGN3479	7.83×10^{-5}	147.6	2.14×10^{-4}	55.1	2.02×10^{-4}	57.3

Source: Table 4, page 20, Study Report REGN3479-MX-15041-sr-01v1.
Abbreviations: EBOV, *Zaire ebolavirus;* GP, glycoprotein; k_d, dissociation rate constant; T½, dissociative half-life; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Binding to sGP. The fourth gene in the EBOV genome encodes two unique proteins, a nonstructural, dimeric sGP, and a trimeric, virion-attached GP. These two GP variants share the first 295 amino acids but have unique C-termini. sGP is the main product transcribed from the GP gene and, its role in EBOV pathogenesis is thought to be diverse but often speculative because the majority of investigations into sGP have been structural studies (de La Vega et al. 2015). sGP has been found in the serum of humans infected with EBOV at concentrations 100- to 1000 fold those of full-length GP (Sanchez et al. 1996; Cook and Lee 2013; de La Vega et al. 2015). The ability of each mAb to bind to recombinant EBOV sGP (sGP.mmh, EBOV Makona) was assessed by interferometry (Octet HTX) (Figure 20). The three REGN-EB3 mAbs showed specific binding to recombinant EBOV Makona GP.10xhis, but only odesivimab (REGN3471) demonstrated specific binding to recombinant EBOV sGP.mmh. The odesivimab (REGN3471) epitope is likely located in a common region within the first 295 amino acids of sGP and EBOV

virion-associated envelope GP; by contrast, the other two mAbs recognize and bind to amino acid residues at the C-terminus of EBOV GP beyond position 295.

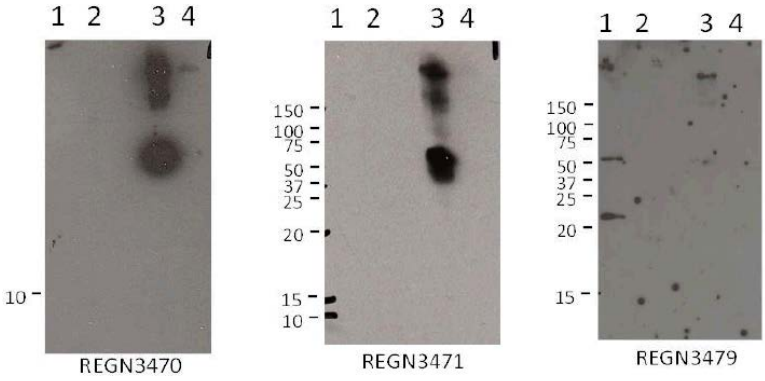
Figure 20. Interaction of Atoltivimab (REGN3470), Odesivimab (REGN3471), and Maftivimab (REGN3479) With EBOV Makona GP.10xhis and EBOV sGP (sGP.mmh) at 25°C[1]



Source: Figure 1, page 21, Study Report REGN3479-MX-15041-sr-01v1
[1] Octet HTX binding signals for each of the anti-EBOV GP antibodies to 300nM EBOV GP.10xhis (blue), EBOV soluble sGP.mmh (red), and the irrelevant, negative control protein hCNTFR.mmh (green). The ordinate designation nm in this figure should be nM.
Abbreviations: EBOV, *Zaire ebolavirus;* GP, glycoprotein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; sGP, secreted glycoprotein

Secondary structural conformation of binding epitopes. Western blot analysis was performed to elucidate the secondary structural conformation of the binding epitopes for the anti-EBOV GP mAbs. The Applicant reported that western blotting showed that the three mAbs bound to recombinant EBOV Makona GP.10xhis, indicating linear epitopes for the three mAbs; however, the signal intensity was markedly lower for maftivimab (REGN3479) (Figure 21). The difference in signal intensity indicates that the epitope for maftivimab (REGN3479) may have a linear component but likely includes nonlinear amino acids proximal to the linear residues.

Figure 21. Detection of Antibody Binding to EBOV GP.10xhis by Western Blot Analysis[1]



Source: Figure 2, page 22, Study Report REGN3479-MX-15041-sr-01v1).
[1] The panels display the binding of REGN3470, REGN3471, and REGN3479 to recombinant EBOV Makona GP.10xhis protein by western blot. For each antibody analyzed, 50 ng (lane 1) or 5 ng (lane 2) of MERS-S purified protein (negative control) and 50 ng (lane 3) or 5 ng (lane 4) of purified EBOV Makona GP.10xhis were reduced with 1× sample reducing reagent and separated on a 4 to 20% Tris-glycine polyacrylamide gel using 1× Tris-glycine-SDS running buffer. Proteins were transferred to a PVDF membrane for western blot analysis using the indicated antibodies.
Abbreviations: EBOV, *Zaire ebolavirus;* GP, glycoprotein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Competition assessment of binding to GP. To determine if atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) possess nonoverlapping binding epitopes on the EBOV GP, a cross-competition assay between paired mAbs was performed by

interferometry (Octet HTX). Following the capture of ~0.5nM of EBOV Makona GP.10xhis protein onto anti-penta-His coated Octet sensors, the assay utilized a format of saturating binding to EBOV Makona GP.10xhis for the first mAb, followed by the binding of a second mAb. All possible combinations were tested using a 3×3 paired matrix. The atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) mAbs do not appear to compete for binding to recombinant EBOV Makona GP.10xhis; and, therefore, are likely to have minimum steric hindrance in binding to the same EBOV GP molecule (Table 94).

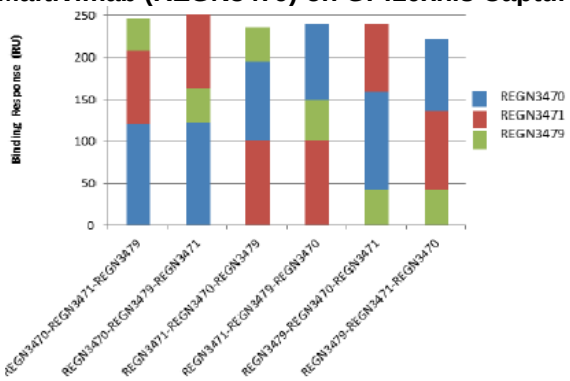
Table 94. Cross-Competition of Atoltivimab (REGN3470), Odesivimab (REGN3471), and Maftivimab (REGN3479) for Binding to EBOV GP.10xhis at 25°C

	Response of 50 µg/mL mAb 2 Binding mAb 1-Bound EBOV GP.10xhis (nm)			
mAb	**REGN3470**	**REGN3471**	**REGN3479**	**REGN1932**
REGN3470	0.04[a]	0.48	0.32	0.03
REGN3471	0.41	0.07[a]	0.31	0.02
REGN3479	0.46	0.44	0.08[a]	0.00
REGN1932[a]	0.52	0.50	0.33	0.02[a]

Source: Table 5, page 23, Study Report REGN3479-MX-15041-sr-01v1.
[a] Represents self-to-self competition.
[b] REGN1932: Nonfucosylated anti-Fel D 1 hIgG1 isotype control antibody.
Abbreviations: EBOV, *Zaire ebolavirus;* GP, glycoprotein; mAb, monoclonal antibody; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Assessment of sequential binding. To further determine if the three mAbs can bind the EBOV GP simultaneously, a three-step, SPR-Biacore sequential binding study was performed. This assay involved injection of saturating amounts of each mAb sequentially over the EBOV Makona GP.10xhis sensor chip surface, using all permutations in the order of antibody injections. Atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) simultaneously bind to the recombinant GP.10xhis (EBOV Makona) surface (Figure 22). The maftivimab (REGN3479) binding signal was lower than that of atoltivimab (REGN3470) and odesivimab (REGN3471) (Figure 22).

Figure 22. Sequential Binding of Atoltivimab (REGN3470), Odesivimab (REGN3471), and Maftivimab (REGN3479) on GP.10xhis Captured Surfaces

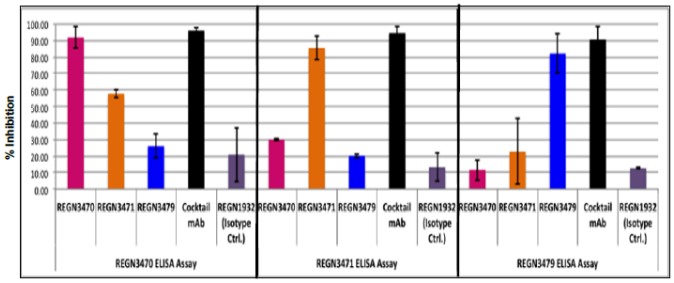


Source: Figure 3, panel B, page 24, Study Report REGN3479-MX-15041-sr-01v1).
Accumulative binding signals for three sequential mAb injections over the EBOV GP.10xhis surface in all permutations of the order of injection. The binding signal of the 50 µg/mL mAb injection is designated as: REGN3470 (blue); REGN3471 (red); and REGN3479 (green) and are stacked in each bar graph with the bottom being the first injection and the top the last injection.
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Concomitant binding assessments. To demonstrate that each individual mAb was functional in binding to the EBOV Makona GP, three separate ELISA-based competition assays were developed by the Applicant and used to determine the functional EBOV Makona GP binding

potency of each anti-EBOV component (atoltivimab [REGN3470], odesivimab [REGN3471], or maftivimab [REGN3479]) of the mAb cocktail. The assays utilized the format of the mAb cocktail blocking EBOV Makona GP.10xhis in solution binding to microtiter plates coated with atoltivimab (REGN3470), odesivimab (REGN3471), or maftivimab (REGN3479). Using varying amounts of the cocktail and a constant concentration (1nM) of EBOV Makona GP.10xhis in solution, the blocking potency of the cocktail was calculated and compared to that of the single mAbs. The half-maximal inhibitory concentration (IC_{50}) values of the mAb cocktail for blocking the binding of recombinant EBOV Makona GP.10xhis to a plate coated with atoltivimab (REGN3470), odesivimab (REGN3471), or maftivimab (REGN3479) were determined to be 28.5nM, 8.74nM, and 10.1nM, respectively (Figure 23). The Applicant stated that the results indicated that the three mAb components (atoltivimab [REGN3470], odesivimab [REGN3471], and maftivimab [REGN3479]) when combined as a mAb cocktail exhibited a similar potency in blocking EBOV Makona GP.10xhis binding to each mAb individually, indicating that the three mAb components of the cocktail were functional in binding to the recombinant EBOV Makona GP.10xhis protein.

Figure 23. Competitive ELISA-Based Assays for Determining the Presence of Atoltivimab (REGN3470), Odesivimab (REGN3471), and Maftivimab (REGN3479) in REGN-EB3



Source: Figure 4, panel D, page 27, Study Report REGN3479-MX-15041-sr-01v1
Percent inhibition of EBOV GP binding at the highest mAb concentration tested for all three ELISA assays. The average data were derived from two independent experiments.
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab.

Conclusions

1. The k_a and k_d rate constants and the K_D equilibrium dissociation constant for the interactions of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) with recombinant EBOV Makona GP.10xhis were determined by SPR-Biacore technology at 25°C and pH 7.4. The results indicated that the three mAbs displayed high-affinity binding to EBOV Makona GP.10xhis. The K_D values for two different lots of each of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479), were 7.74 and 7.93nM, 8.42 and 8.10nM, and 2.97 and 3.70nM, respectively. Atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) dissociation assessed at pH 5.0, 6.0, and 7.4 and 37°C exhibited <2- to 3-fold declines in the dissociation rate constant, indicating that acidic conditions (pH 5) should not have a major effect on the binding kinetics.
2. Interferometry (Octet HTX) was used to determine that odesivimab (REGN3471) specifically binds to sGP (sGP.mmh). However, no appreciable binding of sGP was observed for atoltivimab (REGN3470) or maftivimab (REGN3479).
3. Western blot analysis showed that atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) bind to EBOV Makona GP.10xhis under denaturing and reducing conditions, indicating that each mAb binds to a linear

epitope. However, maftivimab (REGN3479) displayed a significantly lower signal intensity than the other two mAbs, suggesting that additional conformational residues proximal to the linear epitope contribute to binding.

4. mAb cross-competition studies using Octet HTX were performed with atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) in a 3×3 binding matrix. The results indicated the three mAbs do not mutually compete for binding to EBOV Makona GP.10xhis protein, because the three mAbs bound simultaneously.
5. SPR-Biacore experiments were also performed to test the sequential binding of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) to EBOV GP.10xhis protein and the results indicated that these mAbs do not compete for binding and are able to bind EBOV Makona GP.10xhis simultaneously.
6. EBOV GP.10xhis-based blocking ELISAs specific for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) were developed and used to determine the functional EBOV Makona GP binding potency of each anti-EBOV component (atoltivimab [REGN3470], odesivimab [REGN3471], and maftivimab [REGN3479]) of the mAb cocktail. The IC_{50} values of the mAb cocktail for blocking the binding of EBOV Makona GP.10xhis to a plate coated with atoltivimab (REGN3470), odesivimab (REGN3471), or maftivimab (REGN3479) were determined to be 28.5nM, 8.74nM, and 10.1nM, respectively. These values are similar to the IC_{50} values of each single mAb when tested alone in the same assay, indicating that each mAb is functional in binding to the EBOV GP.10xhis protein.

Study Title: Characterization of REGN3470, REGN3471, and REGN3479 Binding to Zaire Ebolavirus Glycoprotein Using Hydrogen-Deuterium Exchange-Mass Spectrometry

Study Report: R3479-PH-20004-SR-01V1

Objective: The objective of the study presented in this report was to characterize the binding of the three individual EBOV GP mAbs of REGN-EB3 to EBOV Makona GP using HDX-MS.

Methods: The binding regions of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) on EBOV Makona GP (EBOV, strain *H.sapiens*-wt/GIN/2014/Kissidougou-C15) were individually characterized by HDX-MS. Amino acid sequence coverages of 68% to 71% were achieved with HDX data. The deuterium uptake percentage (D%) on each peptide was calculated. Peptides that exhibited a difference in D% (calculated as D% on mAb-bound GP minus D% on GP alone) of less than -5% were considered as protected regions. These protected regions were indicative of an antibody-antigen binding region.

Results

Hydrogen-deuterium exchange. HDX-MS is used to map binding regions between mAbs and their target antigens. This method measures the exchange of labile hydrogens, located on the designated antigen backbone, with hydrogens from bulk water. Compared to the free antigen, antibody binding to antigen slows the rate of hydrogen exchange at points of contact between the two proteins and at sites that experience changes in solvent accessibility or structural rigidity. By using heavy water (D_2O) in place of bulk water, rates of exchange can be quantitatively measured by liquid chromatography-mass spectrometry after proteolytic digestion, to map antibody-antigen binding (Ehring 1999; Engen and Smith 2001).

Epitope mapping. The binding regions of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) on EBOV Makona GP were individually characterized by HDX-MS. Amino acid sequence coverages ranging from 68% to 71% were achieved with HDX data. Residues protected by atoltivimab (REGN3470) clustered into three peptide segments: residues 236 to 244 (VDNLTYVQL), 264 to 287 (GKRSNTTGKLIWKVNPEIDTTIGE), and 298 to 308 (TRKIRSEELSF). These three segments span the boundary between the region shared by sGP and GP (residue 295 and before) and the region unique to GP (after residue 295) (Table 95). Residues protected by odesivimab (REGN3471) clustered into four peptide segments: 114 to 122 (KKPDGSECL), 139 to 151 (HKVSGTGPCAGDF), 236 to 244 (VDNLTYVQL), and 265 to 287 (KRSNTTGKLIWKVNPEIDTTIGE). These four segments are contained within the region shared by sGP and GP (Table 95). Residues protected by maftivimab (REGN3479) included one peptide segment: residues 531 to 545 (WIPYFGPAAEGIYTE) (Table 95). This segment is contained within the region unique to GP. These results were consistent with the Bio-Layer Interferometry data that showed that atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) bound recombinant EBOV GP, but only odesivimab (REGN3471) bound recombinant sGP.

Table 95. EBOV GP Peptides Protected by REGN-EB3 mAbs

mAb	Binding Footprint
REGN3470	236 to 244, 264 to 287, 298 to 308
REGN3471	114 to 122, 139 to 151, 236 to 244, 265 to 287
REGN3479	531 to 545

Source: DAV Analyses
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

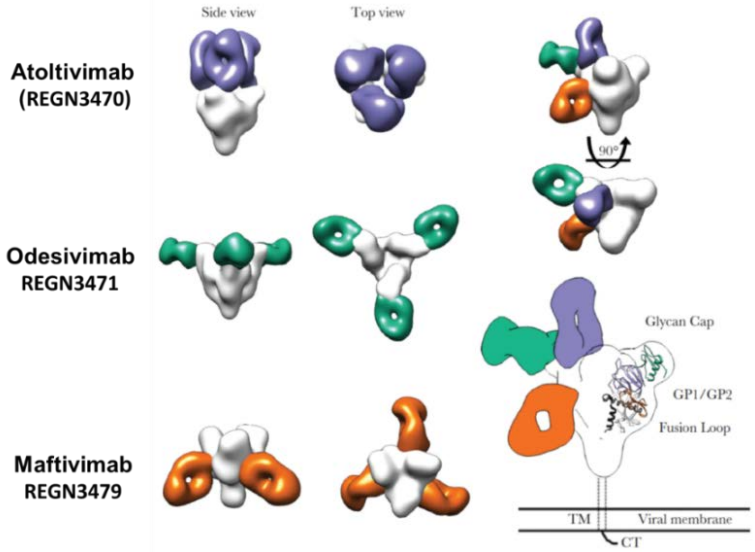
Reviewer's note: The binding footprints for atoltivimab (REGN3470) and odesivimab (REGN3471) overlap at GP residues 236 to 244 and 265 to 287; however, there was no competition noted in the results from the mAb cross-competition studies using Octet HTX shown above. This will be addressed in a PMR/PMC.

Electron microscopy reconstructions. A report of characterization of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) using single-particle, negative-stain electron microscopy (EM) to analyze how Fabs prepared for each mAb bound to the EBOV GP was reviewed to gain insight into the mechanism of action of each of the three mAbs (Pascal et al. 2018). The following characteristics were described:

- **Atoltivimab (REGN3470):** Atoltivimab (REGN3470) binds the EBOV GP parallel to the viral surface on the outside of the glycan cap, at a position that the authors believe explains the neutralization by atoltivimab (REGN3470) of FcγRIIIa activation.
- **Odesivimab (REGN3471):** Odesivimab (REGN3471) binds perpendicular to the viral surface at an angle of approximately 90 degrees. The predicted epitope lies within the chalice structure of the trimer at or near the head of the GP, in an area that likely overlaps with the glycan cap. Each GP trimer can accommodate three odesivimab (REGN3471) Fabs
- **Maftivimab (REGN3479):** Maftivimab (REGN3479) binds at the base of GP, between protomers of GP1/GP2, with the angle of approach being slightly upward from the viral surface. The putative epitope lies near the internal fusion loop and cathepsin cleavage site. The authors speculate that the neutralization activity of maftivimab (REGN3479) may be due to blockade of cathepsin cleavage or release of the fusion loop upon receptor binding.

The EM images were used to generate putative reconstructions of how and where each mAb interacts with the EBOV GP (Figure 24).

Figure 24. Atoltivimab (REGN3470), Odesivimab (REGN3471), and Maftivimab (REGN3479) Bind to Separate Putative Epitopes on the EBOV GP



Source: Figure 1c from (Pascal et al. 2018).
Abbreviations: EBOV, *Zaire ebolavirus;* GP, glycoprotein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Conclusions

- The regions of EBOV Makona GP that were protected during binding of the individual EBOV GP mAbs of REGN-EB3 were identified using HDX-MS and identify putative areas of antibody-antigen contact.
- Atoltivimab (REGN3470) binds the EBOV Makona GP parallel to the viral surface on the outside of the glycan cap, in three putative epitope segments: residues 236 to 244 (VDNLTYVQL), 264 to 287 (GKRSNTTGKLIWKVNPEIDTTIGE), and 298 to 308 (TRKIRSEELSF).
- Odesivimab (REGN3471) binds perpendicular to the viral surface at an angle of approximately 90 degrees. The predicted epitope lies within the chalice structure of the trimer at or near the head of the GP, in an area that likely overlaps with the glycan cap. Odesivimab (REGN3471) binds to both GP and sGP in putative epitope segments: 114 to 122 (KKPDGSECL), 139 to 151 (HKVSGTGPCAGDF), 236 to 244 (VDNLTYVQL), and 265 to 287 (KRSNTTGKLIWKVNPEIDTTIGE).
- Maftivimab (REGN3479) binds at the base of GP, between protomers of GP1/GP2, with the angle of approach being slightly upward from the viral surface. The putative epitope lies near the internal fusion loop and cathepsin cleavage site in one putative epitope segment: residues 531 to 545 (WIPYFGPAAEGIYTE).
- Of note, the binding footprints for atoltivimab (REGN3470) and odesivimab (REGN3471) overlap at GP residues 236 to 244 and 265 to 287; however, the mAb cross-competition studies using Octet HTX yielded no evidence of competition.

Mechanism of Action Conclusions for REGN-EB3

The Applicant provided two nonclinical virology study reports describing experiments performed to assess the mechanisms of action of the three mAbs of the REGN-EB3 cocktail. The results of these experiments are summarized in Table 96. Overall, the data show that all three mAbs bind with high affinity to GP, with odesivimab (REGN3471) also binding sGP. All three mAbs bind to GP simultaneously and sequentially, and binding is not substantially impacted by lower pH. Single-particle, negative-stain EM showed the general areas that each mAb binds, and HDX-MS identified putative epitopes (footprints) for each.

Table 96. REGN-EB3 Mechanism of Action Data (DAV Analyses)

mAb	Binding (2 lots) nM	Binds sGP	Binding GP		Block GP Binding IC_{50} nM	Epitope (Western blot)	Binding Region	Footprint region
			Sim	Seq				
REGN 3470	7.74 and 7.93	No	Yes	Yes	28.5	Linear	Parallel to the viral surface on the outside of the glycan cap	236-244: VDNLTYVQL 264-287: GKRSNTTGKLIW KVNPEIDTTIGE 298-308: TRKIRSEELSF
REGN 3471	8.42 and 8.10	Yes	Yes	Yes	8.74	Linear	Perpendicular to the viral surface within the chalice structure of the trimer at or near the head of the GP in an area that likely overlaps with the glycan cap	114-122: KKPDGSECL 139-151: HKVSGTGPCAGD F 236-244: VDNLTYVQL 265-287: KRSNTTGKLIWKV NPEIDTTIGE
REGN 3479	2.97 and 3.70	No	Yes	Yes	10.1	Linear, con-formational	Base of GP between GP1/GP2, near the internal fusion loop and cathepsin cleavage site	531-545: WIPYFGPAAEGIY TE

The footprint region indicates residues shielded by the mAb in the HDX experiment, suggesting an interaction with the mAb.
Abbreviations: IC_{50}, half maximal inh bitory concentration; mAb, monoclonal antibody; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab.; sGP, secreted glycoprotein; Sim, simultaneously; Seq, sequentially.

The mechanisms of action have not been completely defined for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479).

Questions to be Addressed in a PMR

- What are the epitopes of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479)?
- Does the sGP binding of odesivimab (REGN3471) diminish its antiviral activity and that of the REGN-EB3 cocktail overall?
- Does cross-resistance occur between atoltivimab (REGN3470) and odesivimab (REGN3471), given that the two binding footprints for these mAbs overlap at GP residues 236 to 244 and 265 to 287.

Proposed postmarketing actions:

1. **PMR #1:** Conduct a phenotypic study of the binding and antiviral activity of REGN-EB3, atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) using lentivirus-based particles pseudotyped with EBOV GP containing the substitutions I274M, W275L, G528R, I544T, H549R, N563T, and E564A.
2. **PMR #2:** Conduct a study to characterize genotypically and phenotypically known atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) resistant variants and those identified in cell culture-based escape studies using vesicular stomatitis virus (VSV)-based chimeric virus in binding, neutralization, and ADCC assays with the individual mAbs (atoltivimab [REGN3470], odesivimab [REGN3471], and maftivimab [REGN3479]) and REGN-EB3. In addition, assess cross-resistance between atoltivimab (REGN3470) and odesivimab (REGN3471) using an ADCC assay.
3. **PMC #1**: Please define the precise epitopes of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479).
4. **PMC #2:** Please perform complete resistance analysis of sequences derived from subjects treated with REGN-EB3 in the PALM trial, if these data become available to you in the future.

18.1.2.2. Cell Culture Studies

The Applicant provided two nonclinical virology study reports describing cell culture antiviral activity experiments for the three mAbs in the REGN-EB3 cocktail.

Study Title: In Vitro Characterization of REGN3470, REGN3471, and REGN3479

Study Report: REGN3479-MX-15042-SR-01V2

Objectives: The objectives of this study were:

1. To measure the neutralization of EBOV VLPs by atoltivimab (REGN3470), odesivimab (REGN3741), maftivimab (REGN3749), and REGN-EB3 cocktail in cell culture.
2. To measure the neutralization of infectious EBOV by atoltivimab (REGN3470), odesivimab (REGN3741), and maftivimab (REGN3749) in cell culture.
3. To evaluate the potential of atoltivimab (REGN3470), odesivimab (REGN3741), maftivimab (REGN3749), and the REGN-EB3 cocktail to induce ADCC by monitoring for mAb-induced signaling in Jurkat/FcγRIIIa/NFAT-Luc effector cells in the presence of EBOV GP-engineered target cells.
4. To evaluate the potential of atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), and the REGN-EB3 cocktail to form circulating immune complexes and bind the complement C1q protein, to initiate complement-dependent cytotoxicity.

Neutralization Results

The REGN-EB3 mAbs were assessed for their ability to neutralize EBOV infection in a replication-competent virus infection assay in Vero cells against four EBOV strains (Mayinga, Makona, Kikwit, and Guinea pig-adapted Mayinga). Maftivimab (REGN3479) was able to neutralize the four strains of EBOV based on plaque reduction neutralization test (PRNT) 50% and 80% titers (PRNT-50 and PRNT-80) of 0.1 to 0.2nM and 0.2 to 1.2nM, respectively,

compared to PRNT-50 values of 1.3 to 5.2nM for KZ52, a well characterized mAb against EBOV that binds at the base of GP (Table 97). The neutralization titer was >10-fold lower for maftivimab (REGN3479) against the human EBOV strains compared to a human antibody (KZ52) that neutralizes EBOV infection in cell culture (Parren et al. 2002). Atoltivimab (REGN3470) and odesivimab (REGN3471) did not demonstrate neutralizing activity in this assay.

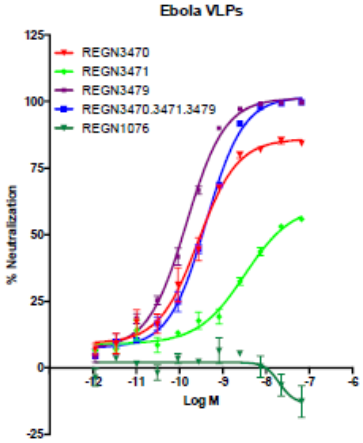
Table 97. Neutralization Titers of Anti-EBOV GP Antibodies in a Replication-Competent EBOV Infection Assay

	Guinea Pig Virus[a]		Kikwit Virus		Makona Virus		Mayinga Virus	
Antibody	PRNT-50 (nM)	PRNT-80 (nM)	PRNT-50 (nM)	PRNT-80 (nM)	PRNT-50 (nM)	PRNT-80 (nM)	PRNT-50 (nM)	PRNT-80 (nM)
REGN3470	NA[b]	NA	NA	NA	NA	NA	NA	NA
REGN3471	NA	NA	NA	NA	NA	NA	NA	NA
REGN3479	0.2	0.6	0.1	0.3	0.2	1.2	0.1	0.2
Isotype Control	NA	NA	NA	NA	NA	NA	NA	NA
KZ52[c]	1.3	13.3	4.7	x[d]	5.2	x	2.0	x

Source: Table 3, page 19, Study Report REGN3479-MX-15042-SR-01V2.
[a] Guinea pig-adapted Mayinga EBOV
[b] NA- no activity
[c] Positive control human neutralizing ant body
[d] x-values could not be calculated
Abbreviations: PRNT, plaque reduction neutralization titer; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab.

The REGN-EB3 mAbs were also tested in Huh7 cell culture for neutralization of EBOV Makona VLPs (Figure 25).

Figure 25. Neutralization of EBOV VLPs in Huh7 Cells



Source: Modified from Figure 2, page 20, Study Report REGN3479-MX-15042-SR-01V2
Abbreviations: EBOV, *Zaire ebolavirus;* REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; VLP, virus-like particle

Atoltivimab (REGN3470) and maftivimab (REGN3479) neutralized EBOV VLPs with half-maximal effective concentration (EC_{50}) values of 0.27nM and 0.14nM, respectively (Table 98). Of note, while neutralization of VLPs was observed with atoltivimab (REGN3470) in this VLP-based assay, no neutralization activity was observed in the replication competent virus assay for this mAb (Table 98), possibly due to different conformations of EBOV GP on infectious EBOV versus EBOV VLPs. Odesivimab (REGN3471) displayed very weak EBOV VLP neutralization, in agreement with the lack of neutralization activity in the live virus assay (Table 98). The

REGN-EB3 mAb cocktail demonstrated neutralization of EBOV VLPs with an EC_{50} value of 0.41nM. To ensure that neutralization was specific for EBOV VLPs, neutralization of MERS-CoV and VSV pseudotyped VLPs was also tested. As expected, none of the antibodies had activity in these assays (Table 98).

Table 98. Determination of EC_{50} Value for Cell Culture Neutralization of EBOV VLPs With Anti-EBOV GP mAbs

Antibody	EC_{50} value (nM)
REGN3470	0.27
REGN3471	X[a]
REGN3479	0.14
REGN-EB3	0.41
REGN1076 (IgG1 Control)	NA[b]

Source: Modified version of Table 4, page 21, Study Report REGN3479-MX-15042-SR-01V2
[a] x-value could not be calculated; EBOV
[b] NA = no activity.
Abbreviations: EC_{50}, half maximal effective concentration; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab.

Effector Function Results

ADCC Signaling Activity. The Applicant reported that ADCC is mediated by clustering and activation of FcγRIIIa on effector cells in the presence of antibody-opsonized target cells. The Applicant used HEK293/Tet-on EBOV Makona GP cells to show that atoltivimab (REGN3470), odesivimab (REGN3471), and the REGN-EB3 cocktail induced dose-dependent activation of FcγRIIIa signaling in Jurkat/NFATLuc/FcγRIIIa ^{176}Val effector cells. In this surrogate cell culture-based assay of potential ADCC activity, atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 mAb cocktail bound to EBOV Makona GP-expressing target cells and induced avidity-driven Fc binding to FcγRIIIa on effector cells, activating signaling with EC_{50} values of 2.9nM, 1.6nM, and 1.7nM, respectively (Table 99). Maftivimab (REGN3479) did not mediate activation of FcγRIIIa signaling up to the highest concentration assessed (40nM), as with other mAbs that bind the base of GP (Davidson et al. 2015).

Table 99. Determination of EC_{50} Values for ADCC Signaling Induction in Jurkat/NFAT-Luc/FcγRIIIa ^{176}Val Effector Cells

	Target Cell Line	
	HEK293/Tet-on/ EBOV GP	HEK293/Tet-on
Antibody	EC_{50} Value (nM)	
REGN3470	2.9	NA
REGN3471	1.6	NA
REGN3479	NA	NA
REGN-EB3	1.7	NA
REGN1076 IgG1^{NF} Control[a]	NA	NA
REGN1932 IgG1 Control[b]	NA	NA

Source: Table 6, page 26, Study Report REGN3479-MX-15042-SR-01V2
[a] Nonfucosylated isotype control
[b] Nonfucosylated anti-Fel D 1 IgG1 negative control
Abbreviations: EBOV, *Zaire ebolavirus;* EC_{50}, half maximal effective concentration; GP, glycoprotein; N/A, not applicable; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab.

Complement binding. The potential of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) to form circulating immune complexes in cell culture was evaluated by incubating recombinant, soluble EBOV Makona GP (sGP.mmh) with each mAb individually, the REGN-EB3 cocktail, or with an isotype control antibody, REGN1076. Atoltivimab

(REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) were each incubated with EBOV Makona sGP.mmh at a 1:1 molar ratio (50nM antibody: 50nM EBOV sGP) while REGN-EB3 was incubated with EBOV sGP.mmh at a molar ratio of 3:1 (150nM mAb cocktail: 50nM EBOV sGP.mmh). The ability of the resulting mixtures to bind C1q, the first step in the complement activation cascade, was then tested by ELISA. No C1q binding was observed for atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), or REGN-EB3 following testing of immune complex formation between anti-EBOV Makona GP mAbs and EBOV Makona sGP.

Conclusions

- In a replication-competent wild-type virus infection assay in Vero cells, maftivimab (REGN3479) neutralized the Mayinga, Kikwit, and Makona strains of EBOV with PRNT-80 concentrations of 0.2 to 1.2nM. Atoltivimab (REGN3470) and odesivimab (REGN3471) did not neutralize EBOV in this assay.
- Atoltivimab (REGN3470), maftivimab (REGN3479), and REGN-EB3 were able to neutralize EBOV VLPs with EC_{50} values of 0.27nM, 0.14nM, and 0.41nM, respectively. In contrast, odesivimab (REGN3471) displayed only very weak neutralization activity. Of note, atoltivimab (REGN3470) exhibited neutralization activity in this VLP-based assay, but no neutralization activity was observed in the replication-competent virus assay for this mAb, possibly due to different conformations of EBOV GP on infectious EBOV versus EBOV VLPs.
- Effector function activity of REGN-EB3 antibodies was assessed with an EBOV Makona GP-expressing cell line and Jurkat/NFAT-Luc/FcγRIIIa reporter effector cells. The EC_{50} values of atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 were 2.9nM, 1.6nM, and 1.7nM, respectively, whereas maftivimab (REGN3479) did not exhibit any FcγRIIIa signaling activity at the maximum concentration tested (40nM).
- No C1q binding was observed for atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), or REGN-EB3 following testing of immune complex formation between anti-EBOV GP mAbs and Ebola sGP.

Study Title: Assessment of the Capacity of REGN3470, REGN3471, and REGN3479 to Mediate Antibody-Dependent Cellular Phagocytosis In Vitro

Study Report: R3479-PH-20055-SR-01V1

Objectives: The objective of the study presented in this report was to assess the ability of atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), and the REGN-EB3 mAb cocktail to mediate Fc gamma receptor 3A (FcγR3A)-dependent antibody-dependent cellular phagocytosis (ADCP) of Raji cells engineered to express EBOV Makona GP.

Experimental Methods

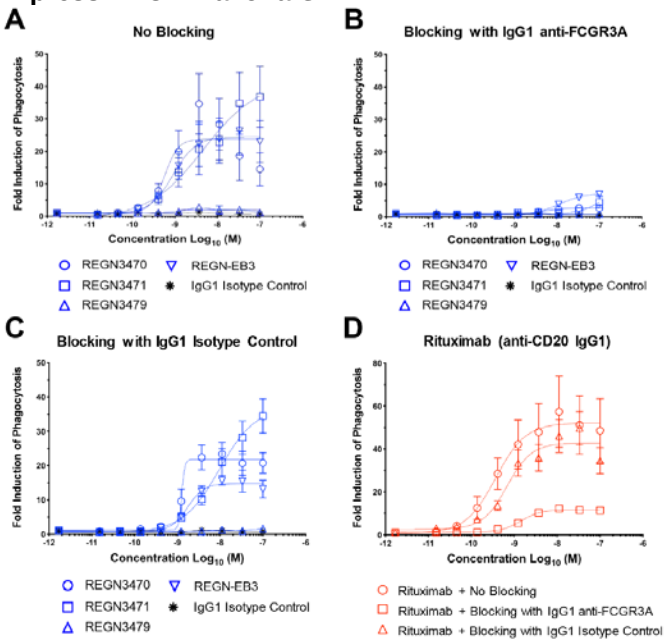
Cells. This experiment used Raji B-cells (ATCC® CCL-86™) genetically engineered to induce the expression of full-length EBOV (Zaire 2014) GP (M1 to F676 of accession #QDA39862.1) upon treatment with doxycycline as the target cells (Raji/Tet-On/EBOV Makona GP). This experiment used human peripheral blood $CD14^+$ monocytes as the effector cells. The EBOV (Zaire 2014) GP originated from the EBOV Makona variant from isolate *H.sapiens*-wt/GIN/2014/Makona-Kissidougou-C15 and will be labeled as EBOV Makona throughout this review.

Experimental design. An ADCP assay was performed to assess the ability of atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), and the REGN-EB3 mAb cocktail to mediate FcγR3A-dependent ADCP. Raji target cells expressing the EBOV Makona GP were exposed to 15pM to 100nM of each independent mAb of the REGN-EB3 cocktail or a positive, negative, or no-antibody control; and phagocytosis was assessed in the presence of phagocytes differentiated from human primary $CD14^+$ monocytes in the presence of GM-CSF. Phagocytosis was measured by fluorescence imaging comparing target cell excitation at 488 nm (carboxyfluorescein diacetate succinimidyl ester–labeled target cells) to phagocyte excitation at 647 nm (Far-Red-labelled macrophages). An IgG1 isotype control was evaluated in parallel with the anti-EBOV GP mAbs. Rituximab (anti-CD20 IgG1 mAb) was used as a positive control for inducing ADCP of target cells. The maximum fold induction of phagocytosis above background (no antibody control) and EC_{50} values were reported.

Results

Atoltivimab (REGN3470), odesivimab (REGN3471), and the REGN-EB3 mAb cocktail each mediated ADCP of Raji/Tet-On/EBOV Makona GP target cells in a concentration-dependent manner with EC_{50} values of 578pM, 4.71nM, and 904pM, respectively (Figure 26).

Figure 26. Anti-EBOV GP mAbs Mediate FcγR3A-Dependent ADCP of Raji Cells Engineered to Express EBOV Makona GP



Source: Figure 1, page 12, Study Report R3479-PH-20055-SR-01V1
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab.

The maximum fold induction of phagocytosis was similar by atoltivimab (REGN3470), odesivimab (REGN3471), and the REGN-EB3 mAb cocktail (26.4- to 36.8-fold over background) (Table 100). No ADCP activity was observed with maftivimab (REGN3479) or the IgG1 isotype control. ADCP activity mediated by atoltivimab (REGN3470), odesivimab (REGN3471), and the REGN-EB3 mAb cocktail was blocked by preincubation of phagocytes with an anti-FcγR3A mAb but not an IgG1 isotype control mAb (Table 100).

Table 100. Summary of REGN-EB3 mAb-Mediated ADCP of Raji Cells Engineered to Express EBOV Makona GP

	No Blocking		Blocking with IgG1 Anti-FCGR3A		Blocking with IgG1 Isotype Control	
Antibody	**EC_{50} (M)**	**Max Fold Induction of Phagocytosis***	**EC_{50} (M)**	**Max Fold Induction of Phagocytosis***	**EC_{50} (M)**	**Max Fold Induction of Phagocytosis***
REGN3470	5.78E-10	34.7	4.38E-09	2.7	1.27E-09	23.4
REGN3471	4.71E-09	36.8	NC	4.6	9.56E-09	34.5
REGN3479	ND	2.8	ND	1.3	ND	1.6
REGN-EB3	9.04E-10	26.4	1.17E-08	6.9	1.73E-09	15.7
IgG1 Isotype Control	ND	1.3	ND	1.2	ND	1.2
Rituximab (anti-CD20 IgG1)	3.69E-10	57.4	1.43E-09	12.1	6.79E-10	49.7

Source: Table 2, page 14, Study Report R3479-PH-20055-SR-01V1
Abbreviations: EBOV, *Zaire ebolavirus;* EC_{50}, half maximal effective concentration; GP, glycoprotein; IgG1, immunoglobulin G1; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab.

Conclusions

The results of this study indicate that atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 induce FcγR3A-mediated ADCP of target cells expressing EBOV Makona GP with EC_{50} values of 578pM, 4.71nM, and 904pM, respectively. Maftivimab (REGN3479) did not induce FcγR3A-mediated ADCP of target cells at concentrations up to 100nM. This information will be added to the label.

Cell Culture Antiviral Activity Conclusions for REGN-EB3

The Applicant provided two nonclinical virology study reports describing cell culture antiviral activity experiments for the three mAbs in the REGN-EB3 cocktail. The results of these experiments are summarized in Table 7. Overall, the data show that maftivimab (REGN3479) and REGN-EB3 neutralized four replication-competent EBOV strains in Vero cells with PRNT-80 titers of 0.2 to 1.2nM; atoltivimab (REGN3470), maftivimab (REGN3479), and REGN-EB3 neutralized EBOV VLPs with EC_{50} values of 0.27nM, 0.14nM, and 0.41nM, respectively; and ADCC signaling through the FcγRIIIa pathway was characterized for atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 with EC_{50} values of 2.9nM, 1.6nM, and 1.7nM, respectively. Binding of C1q to initiate complement activation was not detected for any mAb. Atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 induced FcγR3A-mediated ADCP of target cells expressing EBOV Makona GP with EC_{50} values of 578pM, 4.71nM, and 904pM, respectively. Maftivimab (REGN3479) did not induce FcγR3A-mediated ADCP of target cells at concentrations up to 100nM Table 7. ***Fc-effector function.*** Atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 induce FcγR3A-mediated ADCP of target cells expressing EBOV Makona GP with EC_{50} values of 578pM, 4.71nM, and 904pM, respectively. Maftivimab (REGN3479) did not induce FcγR3A-mediated ADCP of target cells at concentrations up to 100nM.

Considerations for postmarketing actions: Determine the impact of atoltivimab (REGN3470) resistance substitutions selected in a neutralization assay on Fc effector functions.

Proposed PMR: Conduct a study to characterize genotypically and phenotypically known atoltivimab (REGN3470)-, odesivimab (REGN3471)-, and maftivimab (REGN3479)-resistant variants and those identified in cell culture-based escape studies using VSV-based chimeric virus in binding, neutralization, and ADCC assays with the individual mAbs (atoltivimab [REGN3470], odesivimab [REGN3471], and maftivimab [REGN3479]) and the REGN-EB3 combination. In addition, assess cross-resistance between atoltivimab (REGN3470) and odesivimab (REGN3471) using an ADCC assay.

18.1.2.3. Resistance and Cross-Resistance Assessments in Cell Culture

Resistance Assessments in Cell Culture

No cell culture selection experiments were performed to identify amino acid substitutions in EBOV GP that lead to reduced susceptibility to each mAb in the REGN-EB3 cocktail. This deficiency will be addressed with postmarketing actions.

Cross-Resistance Assessments in Cell Culture

PREVAIL II (NCT02363322) was a randomized, controlled trial designed to assess the efficacy of ZMapp™ (a cocktail of three mAbs directed against EBOV GP) plus the current standard of care compared to the current standard of care alone in subjects with EBOV infection in West Africa (PREVAIL II Writing Group 2016). Although the estimated effect of ZMapp™ against EBOV infection appeared to be beneficial, the clinical trial was unable to enroll enough subjects and so did not meet the prespecified statistical threshold for efficacy (PREVAIL II Writing Group 2016). However, based on the results of PREVAIL II, ZMapp™ was used as the standard of care in protocols assessing the impact of investigational products during the EBOV outbreak that began in the DRC in August 2018 (Mulangu et al. 2019). As a result, Clinical Virology requested that the Applicant assess substitutions in GP that had been associated with escape from ZMapp™ mAbs (Qiu et al. 2012; Audet et al. 2014; Murin et al. 2014; Davidson et al. 2015). The substitutions of interest were GP I274M and W275L, which are in the 13c6 epitope, and Q508R, which impacts mAbs binding at the base of GP, 2G4, and 4G7 (Davidson et al. 2015).

The Applicant provided a study report showing assessments of Q508R, which is reviewed below; however, they did not assess I274M and W275L. Subsequent to the submission of that study report, the Applicant provided Study Report R3479-PH-20004-SR-01V1 wherein they define the binding footprints for the three REGN-EB3 mAbs (Section 18.1.2.1). The binding footprint for maftivimab (REGN3479) was identified as a peptide segment corresponding to GP residues 531 to 545, which is distinct from the Q508 position associated with resistance to ZMapp™. Of note, I274M and W275L fall within the binding footprints of atoltivimab (REGN3470) and odesivimab (REGN3471). Characterization of I274M and W275L on the binding and antiviral activity of atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3 will be communicated as a postmarketing requirement related to characterizing the resistance pathways for REGN-EB3.

Study Title: Properties of REGN3470, REGN3471, and REGN3479 on Ebola Virus Glycoprotein ZMapp™ Escape Mutant

Study Number: REGN3479-MX-16084-SR-01V1

Objectives: The objective of this study was to measure the ability of atoltivimab (REGN3470), odesivimab (REGN3741), maftivimab (REGN3749), and REGN-EB3 to neutralize EBOV

Kikwit VLPs containing the ZMapp™ escape substitution at position Q508 in the EBOV Kikwit GP (Audet et al. 2014; Davidson et al. 2015).

Test System

Atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), and REGN-EB3 were analyzed for their ability to neutralize EBOV Kikwit VLPs in a cell culture-based assay. VLPs expressing EBOV Kikwit GP were incubated with serial dilutions of antibody for 1 hour and then added to Huh7 cells (human hepatoma cells). At 72 hours postinfection, the infection efficiency was quantitated by detection of luciferase.

Experimental Design

Site-directed mutagenesis of EBOV GP-Kikwit 1995 strain. Site-directed mutagenesis (QuikChange II Site Directed Mutagenesis kit; Agilent, Santa Clara, CA, USA) of the GP of the EBOV Zaire 1995 strain was performed using primers (Integrated DNA Technologies, Coralville, IA, USA) to change the glutamine at position 508 to arginine (Q508R). The EBOV Kikwit 1995 GP sequence was confirmed by Sanger sequencing using a 3739x1DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and the entire GP was subcloned into the pRG984 expression vector.

EBOV virus-like particle-based neutralization assay. EBOV GP pseudoparticles (VLPs) were generated by cotransfecting 293T cells with a mix of plasmid constructs expressing EBOV Makona 2014 strain or Kikwit 1995 strain-Q508R GP, HIV gag-pol, and an HIV proviral vector encoding firefly luciferase. Supernatants containing EBOV GP pseudoparticles were harvested at 48 hours post-transfection, clarified by centrifugation, aliquoted, and stored frozen at -80°C. The EBOV GP VLPs were tested in neutralization assays in Huh7 cells. Specifically, serial three-fold dilutions of antibody ranging from 2.8pM to 167nM were incubated with EBOV GP VLPs for 1 hour at room temperature and added to Huh7 cells. Infection efficiency was quantitated by BrightGlo® Luciferase Assay (Promega, San Luis Obispo, CA, USA) and relative luminescence units (RLU) were measured using a Victor® X3 Plate Reader (Perkin Elmer, Waltham, MA, USA).

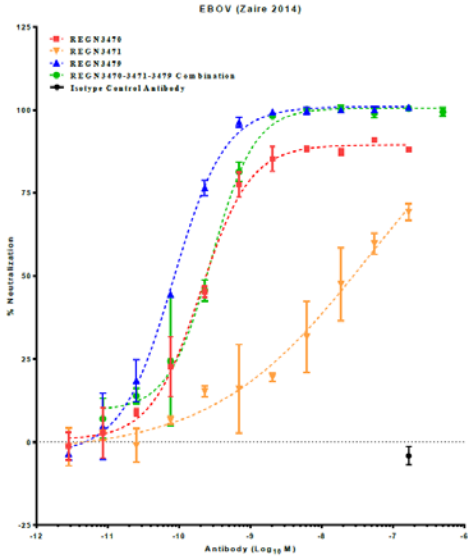
Results

EBOV Makona GP. Concentrations of the anti-EBOV GP mAbs of 2.8pM to 167nM were tested in cell culture for neutralization of EBOV Makona VLPs in Huh7 cells. The Applicant stated that atoltivimab (REGN3470) and maftivimab (REGN3479) were able to neutralize VLPs pseudotyped with the EBOV Makona GP with EC_{50} values of 0.2nM and 0.08nM, respectively (Figure 27; Table 101). The Applicant also reported that odesivimab (REGN3471) displayed very weak EBOV VLP neutralization. The REGN-EB3 mAb cocktail neutralized EBOV VLPs with an EC_{50} value of 0.28nM. The slopes of the VLP neutralization curves indicate that REGN-EB3, atoltivimab (REGN3470), and maftivimab (REGN3479) exhibit strong neutralization potential whereas odesivimab (REGN3471) does not. Of note, the maximum saturation of atoltivimab (REGN3470) neutralization is <100%.

EBOV Kikwit-Q508R GP. The HDX-MS experiments indicated that maftivimab (REGN3479) binds at the base of GP, and the Q508R substitution has been shown to impact other mAbs binding at the base (KZ52, 2G4, and 4G7; (Davidson et al. 2015). The anti-EBOV GP mAbs were further tested in cell culture for their ability to neutralize VLPs generated using EBOV Kikwit GP containing the ZMapp™ Q508R escape substitution. Atoltivimab (REGN3470) and

maftivimab (REGN3479) neutralized VLPs pseudotyped with EBOV Kikwit 1995-Q508R GP with EC_{50} values of 0.19nM and 0.16nM, respectively (Figure 28; Table 101).

Figure 27. Cell Culture Neutralization of EBOV (Makona 2014 Strain) Virus-Like Particles in Huh7 Cells



Source: Figure 1, page 10, REGN3479-MX-16084-SR-01V1)
Anti-EBOV GP mAbs (2.8pM-167nM) were tested for the ability to neutralize EBOV Makona 2014 strain virus-like particles (VLPs).REGN3470 (red square), REGN3471 (inverted orange triangle), and REGN3479 (blue triangle) were tested individually and in combination (REGN3470-3471-3479-green circle) for neutralization of VLPs in Huh7 cells. The isotype control antibody (H1H8339c-human IgG1 anti-Influenza M2 antibody) did not show any neutralization activity of EBOV VLPs in this assay (black circle; 167nM).
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab.

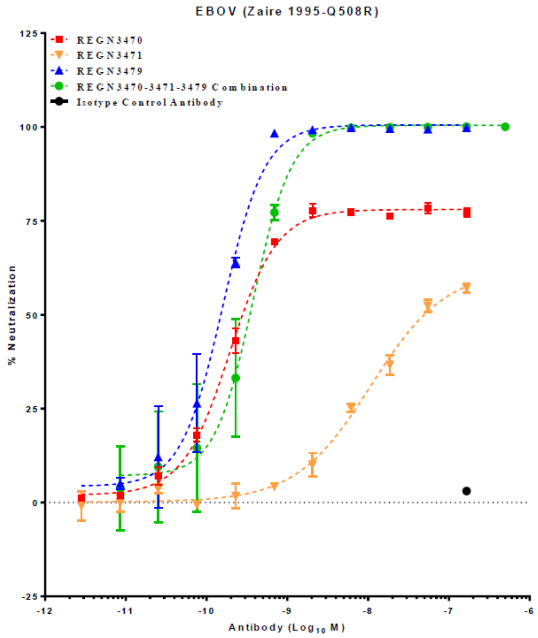
These values were similar to those determined for neutralization of EBOV Makona VLPs. Odesivimab (REGN3471) displayed very weak EBOV Kikwit 1995 VLP neutralization, similar to that for neutralization of EBOV Makona 2014 VLPs. The REGN-EB3 mAb cocktail neutralized EBOV Kikwit 1995 VLPs with an EC_{50} value of 0.38nM (Table 101). These results indicate that the Q508R substitution does not reduce susceptibility to maftivimab (REGN3479), which is consistent with the HDX-MS results reported in Section 18.1.2.1.

Table 101. EC_{50} Values for Cell Culture Neutralization of EBOV VLPs With REGN-EB3 mAbs

	EC_{50} Value (M)		
Antibody	**Makona 2014**	**Kikwit 1995-Q508R**	**Fold Shift**
REGN3470	2.00E-10	1.94E-10	1.0
REGN3471	4.49E-08	1.08E-08	0.2
REGN3479	8.28E-11	1.59E-10	1.9
REGN-EB3	2.84E-10	3.76E-10	1.3

Source: Modified from Table 2, page 13, REGN3479-MX-16084-SR-01V1
Abbreviations: EC_{50}, half maximal effective concentration; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Figure 28. Cell Culture Neutralization of EBOV (Kikwit 1995 Strain-Q508R) Virus-Like Particles in Huh7 Cells



Source: Figure 2, page 12, REGN3479-MX-16084-SR-01V1.
Anti-EBOV GP mAbs (2.8pM-167nM) were tested for the ability to neutralize EBOV (Kikwit 1995 strain) virus-like particles (VLPs) containing the EBOV GP ZMapp™ escape substitution (Q508R). REGN3470 (red square), REGN3471 (inverted orange triangle), and REGN3479 (blue triangle) were tested individually and in combination (REGN3470-3471-3479-green circle) for neutralization of VLPs in Huh7 cells. The isotype control antibody (H1H8339c-human IgG1 anti-influenza M2 antibody) did not show any neutralization activity of EBOV VLPs in this assay (black circle; 167nM).
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

***Caveats*:** The GP sequences used to make VLPs were described in the study report as EBOV Zaire 1995 and 2014, and presumably the 1995 EBOV sequence was an EBOV Kikwit strain and the 2014 virus was an EBOV Makona strain sequence, as in previous submissions from this Applicant. It is not clear why the Applicant compared a mutant in one backbone (EBOV Kikwit 1995) to the wild-type of the other (EBOV Makona 2014).

Conclusions

1. REGN-EB3 had similar neutralization activity against VLPs from EBOV Kikwit and EBOV Makona.
2. The Q508R substitution associated with resistance to ZMapp™ did not confer cross-resistance to REGN-EB3 or any of its constituent mAbs.
3. Consistent with the data in Section 18.1.2.1and Section 18.1.2.2, odesivimab (REGN3471) had the weakest neutralization response of the three mAbs; however, it exhibited greater neutralization activity than the isotype control.
4. The Applicant did not assess the cross-resistance potential of I274M or W275L. Subsequent to the submission of this study report, the Applicant provided Study Report R3479-PH-20004-SR-01V1 wherein they define the binding footprints for the three REGN-EB3 mAbs (See Section 18.1.2.1). Of note, I274M and W275L fall within the binding footprints of atoltivimab (REGN3470) and odesivimab (REGN3471).

Considerations to be addressed in a PMR: Cross-resistance characterization of GP substitutions I274M and W275L and their impact on the binding and antiviral activity of atoltivimab (REGN3470), odesivimab (REGN3471), and REGN-EB3.

1. The impact of resistance-associated substitutions on Fc effector functions should be assessed.

Proposed resistance-related PMRs:

1. **PMR #1:** Conduct a phenotypic study to determine the impact on binding and antiviral activity against REGN-EB3, atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) using lentivirus-based particles pseudotyped with EBOV GP containing the substitutions: I274M, W275L, G528R, I544T, H549R, N563T, and E564A
2. **PMR #2:** Conduct a study to characterize genotypically and phenotypically known atoltivimab (REGN3470)-, odesivimab (REGN3471)-, and maftivimab (REGN3479)-resistant variants and those identified in cell culture-based escape studies using VSV-based chimeric virus in binding, neutralization, and ADCC assays with the individual mAbs (atoltivimab [REGN3470], odesivimab [REGN3471], and maftivimab [REGN3479]) and the REGN-EB3 combination. In addition, assess cross-resistance between atoltivimab (REGN3470) and odesivimab (REGN3471) using an ADCC assay.

18.1.2.4. Animal Studies

Studies in Guinea Pigs

The Applicant submitted two study reports, (b) (4) 2015-001 Set#1 and (b) (4) 2015-001 Set#2, describing studies in an EBOV guinea pig-challenge model. The studies were performed by and the study reports were prepared by the (b) (4) The design of Sets 1 and 2 of the study was similar with the exception that relative to EBOV infection on Day 0, animals were dosed with mAbs 1 day after infection in Set#1 and 3 days after infection in Set#2. In addition, Set#2 tested combinations of mAbs, whereas Set#1 tested only single mAbs.

Study Title: Efficacy (sic) Screening of Filovirus Monoclonal Antibodies in Guinea Pigs

Study Number: (b) (4) 2015-001 Set#1; Day 4 titers

Purpose: To assess individually the antiviral activity of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) in guinea pigs lethally challenged by IP injection with guinea pig-adapted EBOV Mayinga on Day 0, with animals receiving treatment with atoltivimab (REGN3470), odesivimab (REGN3471), or maftivimab (REGN3479) on Day 1. These treatment groups were compared to an irrelevant control antibody and REGN3474, which was a fourth mAb under consideration but not selected for the REGN-EB3 cocktail. The guinea pig adapted EBOV Mayinga master stock originating from a human serum specimen (057931).

Summary: This was a blinded study wherein 30 Hartley guinea pigs were exposed to 4,190 PFU (target of 1,000 PFU by FANG plaque assay) guinea pig-adapted EBOV Mayinga by the IP route on Day 0. Twenty-four hours after exposure, guinea pigs were treated IP (Pascal et al. 2018) with one of five antibody formulations (atoltivimab [REGN3470], odesivimab [REGN3471], REGN3474, maftivimab [REGN3479], and an irrelevant antibody-placebo; Table 102) and observed daily for signs of disease until Day 28 postexposure. Of note, changes in

EBOV-Mayinga that developed during adaptation to guinea pigs resulted in modest reductions in susceptibility (Table 97).

Table 102. Experimental Groups of (b) (4) 2015-001 Set#1

Group	Size	Treatment mAb	Dose
1	6	REGN3470	5 mg
2	6	REGN3471	5 mg
3	6	Placebo	None
4	6	REGN3474	5 mg
5	6	REGN3479	5 mg

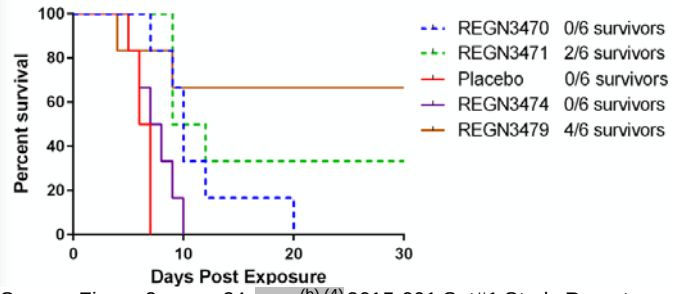
Source: Table 2, page 15, (b) (4) 2015-001 Set#1 Study Report
Abbreviations: mAb, monoclonal antibody; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Body weight and temperature measurements were performed per study plan and plaque assay was conducted to determine viremia on Day 4 per standard procedures. Four of six animals treated with maftivimab (REGN3479) and two of six animals treated with odesivimab (REGN3471) survived the lethal challenge. Animals treated with atoltivimab (REGN3470) and odesivimab (REGN3471) that succumbed to the lethal challenge exhibited a delayed time to euthanasia/death compared to the placebo-treated controls (Figure 29). No significant reduction in time to euthanasia/death compared to placebo was seen for two guinea pigs treated with maftivimab (REGN3479) that died.

Results

Survival analysis. All guinea pigs in the placebo group (received phosphate-buffered saline [PBS] control) succumbed to EBOV infection by Day 7. Atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) delayed EBOV disease and prevented or delayed death during the 3-week observation period (Figure 29). In the maftivimab (REGN3479) treatment group, four of six guinea pigs survived and in the odesivimab (REGN3471) treatment group, two of six guinea pigs survived. There were no survivors in the other groups (Figure 29). Of note, the clinical score, weight, and temperature were provided and reviewed but are not shown here.

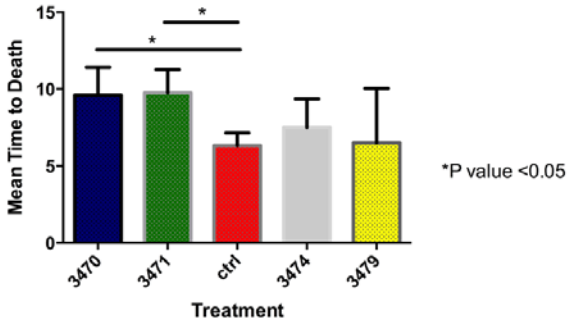
Figure 29. Kaplan-Meier Plot for Survival Analysis of Guinea Pigs Exposed to Guinea Pig Adapted Ebola Virus and Treated With Monoclonal Antibody or Placebo



Source: Figure 3, page 24, (b) (4) 2015-001 Set#1 Study Report.
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

In addition, guinea pigs treated with atoltivimab (REGN3470) and odesivimab (REGN3471) exhibited a delay in time-to-death compared to placebo-treated controls (Figure 29 and Figure 30). No significant difference in time-to-death compared to the placebo control was seen for the two guinea pigs treated with maftivimab (REGN3479) that died (Figure 30).

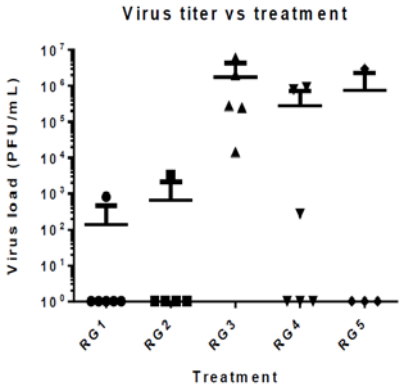
Figure 30. Mean Time to Death for Guinea Pigs Exposed to EBOV by the Intraperitoneal Route per Treatment Formulation



Source: Figure 2, page 24, (b) (4) 2015-001 Set#1 Study Report
Animals that survived were not included in this calculation.
Abbreviations: EBOV, *Zaire ebolavirus*

Viral load analysis. The viral load in samples from all guinea pigs was analyzed by plaque assay at Day 4 (Figure 32). Group 1 (RG1, atoltivimab [REGN3470]) and Group 2 (RG2, odesivimab [REGN3471]) had mean viral load reductions of approximately 4 $\log_{10}$ compared to placebo (RG3).

Figure 31. Viral Load Analysis of (b) (4) 2015-001 Set#1 Viral Load at Day 4



Source: Set1 Day 4 Virus Titer Data
Abbreviations: PFU, plaque-forming unit

Groups 4 and 5 (maftivimab [REGN3479]) had mean viral load reductions of <1 $\log_{10}$ compared to placebo (Figure 32). Of note, virus was not detected by plaque assay and reported as 0 PFU/mL for the four animals treated with maftivimab (REGN3479) that survived infection; the viral titer was also reported as 0 PFU/mL for one animal that died/was euthanized on Day 9. The samples for plaque assays were 500 µL. The lower limit of quantification of the assay was not provided in the study report but it was the standard FANG plaque assay with a reported lower limit of quantitation of 10 to 50 PFU/mL (Shurtleff et al. 2016). Data on RNA genomes/mL were not provided; these could have indicated that virus neutralization was occurring in vivo.

Conclusions

- In a live virus PRNT assay, guinea pig-adapted Mayinga was neutralized by maftivimab (REGN3479) with an EC_{50} value of 0.2nM. Neutralization activity was not detected for atoltivimab (REGN3470) or odesivimab (REGN3471) in this assay (Pascal et al. 2018).
- Thirty Hartley guinea pigs were challenged with 1,000 PFU EBOV-Mayinga by the IP route on Day 0. Twenty-four hours after exposure, guinea pigs were treated with atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), or an

irrelevant antibody-placebo and observed daily for signs of disease until Day 28 postexposure.

- In the maftivimab (REGN3479) treatment group, four of six guinea pigs survived and in the maftivimab (REGN3479) treatment group, two of six guinea pigs survived. There were no survivors in the other groups, including the placebo group.
- Animals treated with atoltivimab (REGN3470) and odesivimab (REGN3471) that died exhibited a delayed time-to-death compared to placebo-treated controls. No significant reduction in time-to-death was seen for the guinea pigs treated with maftivimab (REGN3479) that died as compared to the placebo controls.
- Guinea pigs treated with atoltivimab (REGN3470) and odesivimab (REGN3471) had mean viral load reductions of approximately 4 $\log_{10}$ whereas those treated with maftivimab (REGN3479) had a mean viral load reduction of <1 $\log_{10}$ compared to placebo. Of note, virus was not detected by plaque assay or reported as 0 PFU/mL for the four animals treated with maftivimab (REGN3479) that survived infection; the viral titer was also reported as 0 PFU/mL for one animal that died/was euthanized on Day 9.
- Maftivimab (maftivimab [REGN3479]) was more protective than the other two mAbs (four of six animals survived), but it had less of an impact on viral titer by Day 4. Of note, atoltivimab (REGN3470) and odesivimab (REGN3471), both of which signal through the FcγRIIIa pathway, contain the human Fc and therefore may not interact with guinea pig Fcγ receptors, hampering interpretation of the contribution of Fc-mediated effector activity in this model (Pascal et al. 2018).
- No resistance assessment was performed for animals that failed treatment in this study.

Study Title: Efficacy (sic) Screening of Filovirus Monoclonal Antibodies in Guinea Pigs

Study Number: (b) (4) 2015-001 Set#2; Day 4 titers

Purpose: To assess the antiviral activity of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) in various cocktail formulations in guinea pigs lethally challenged IP with guinea pig-adapted EBOV strain Mayinga on Day 0, with animals receiving treatment IP 3 days after infection. A nonreactive control, REGN3051, was used to maintain three mAbs in each formulation and as a comparison group. The guinea-pig-adapted Ebola virus strain Mayinga master stock originated from a human serum specimen (057931).

Summary: This was a blinded study conducted at (b) (4). For Set #2, 30 male Hartley guinea pigs aged 3 to 6 months were challenged IP with 0.25 mL of guinea pig-adapted EBOV-Mayinga diluted to 7,340 PFU/mL (target of 1,000 PFU) on Day 0. Seventy-two hours after exposure, the 30 guinea pigs, which were randomized into five groups of six animals each, were treated IM with 5 mg of one of five antibody formulations: REGN-EB3, atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051, atoltivimab (REGN3470)-maftivimab (REGN3479)-REGN3051, and odesivimab (REGN3471)-maftivimab (REGN3479)-REGN3051) or a fifth irrelevant antibody control (REGN3051) and observed a minimum of twice daily for signs of disease until Day 28 postexposure (Table 103).

Animals treated with REGN-EB3, atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051, and atoltivimab (REGN3470)-maftivimab (REGN3479)-REGN3051 had a delayed time-to-death compared to the nonreactive antibody-treated control. Each of the treatments resulted in protection of some animals from lethal infection, with 7 of the 24 treated animals surviving to the end of the study.

Table 103. Experimental Groups of (b) (4) 2015-001 Set#2

Group	Size	Treatment mAb	Dose
RG6	6	REGN-EB3	5 mg
RG7	6	REGN3470, REGN3471, REGN3051	5 mg
RG8	6	REGN3051 (Placebo)	None
RG9	6	REGN3470, REGN3479, REGN3051	5 mg
RG10	6	REGN3471, REGN3479, REGN3051	5 mg

Source: Table 2, Page 15, (b) (4) 2015-001 Set#2 Study Report
Abbreviations: mAb, monoclonal antibody; REGN3051, a nonreactive control mAb. REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

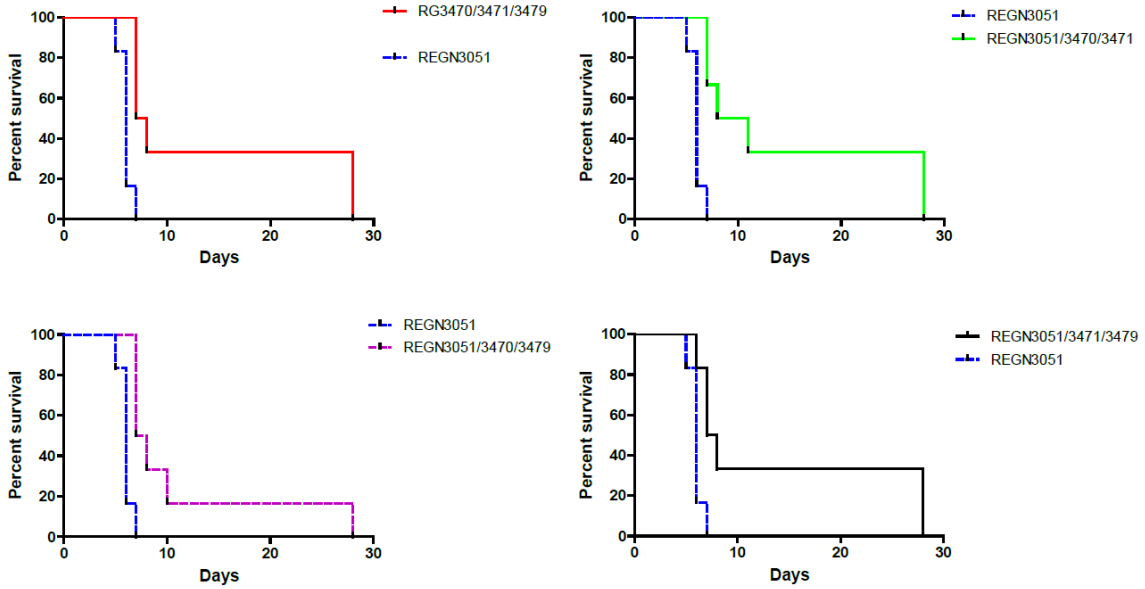
Results

On Day 4, blood was collected for virology, hematology, and clinical chemistry assessments. Animals were weighed daily beginning at Day -1 prior to virus exposure and continued daily until 14 days postexposure. In addition, animals were weighed three times weekly from Day 15 until the end of the study on Day 28.

Survival analysis. Each of the mAb cocktail formulations resulted in protection of animals from lethal infection, with 7 of the 24 treated animals surviving to the end of the study (Day 28) (Figure 32). Animals 34787 and 34789 from the REGN-EB3 group survived until the end of the observation period (Day 28), as did animals 34780 and 34797 in the atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051 group; animal 34786 in the atoltivimab (REGN3470)-maftivimab (REGN3479)-REGN3051 group; and animals 34778 and 34782 in the odesivimab (REGN3471)-maftivimab (REGN3479)-REGN3051 group.

Animals treated with atoltivimab (REGN3470)-odesivimab (REGN3471)-maftivimab (REGN3479), atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051, and atoltivimab (REGN3470)-maftivimab (REGN3479)-REGN3051 exhibited a delayed time-to-death compared to the nonreactive antibody-treated controls (Figure 32).

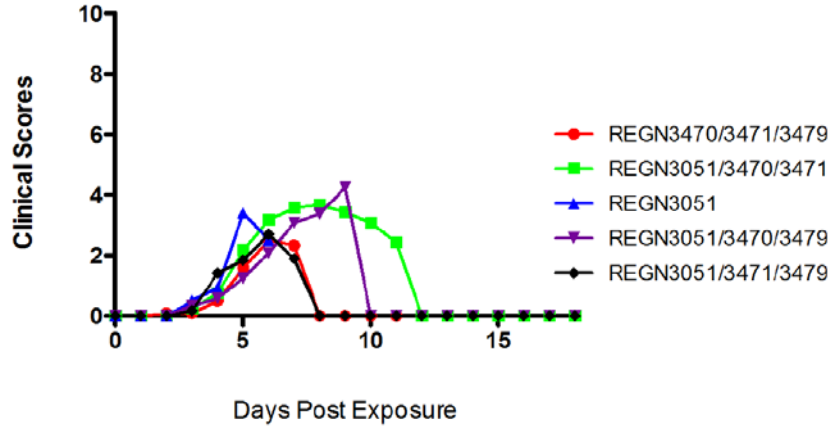
Figure 32. Kaplan-Meier Plot for Survival of Guinea Pigs Exposed to Guinea Pig-Adapted Ebola Virus and Treated With Different mAb Cocktail Formulations



Source: Figure 3, Page 24, (b) (4) 2015-001 Set#2 Study Report
Abbreviations: mAb, monoclonal antibody; REGN3051, a nonreactive control mAb. REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Clinical score assessments. From days 3 to 5 postviral challenge, all animals in the placebo-treated group developed severe clinical signs of disease and by days 5 to 7 succumbed to infection. Animals treated in all cohorts except the placebo control group showed a similar pattern of onset of clinical symptoms beginning on days 3 to 5 (Figure 33). Three animals (34871, 34779, and 34783) treated with REGN-EB3 died on Day 7 after exhibiting signs of weight loss, increased temperature, decreased activity and response, decreased feed consumption, stool production, and fluid intake; and animal 34784 died on Day 9. Animal 34783 was found dead in cage (FDIC) on Day 7. Animals treated with REGN-EB3 and odesivimab (REGN3471)-maftivimab (REGN3479)-REGN3051 showed lower clinical scores after Day 5 compared to those in the other treatment groups.

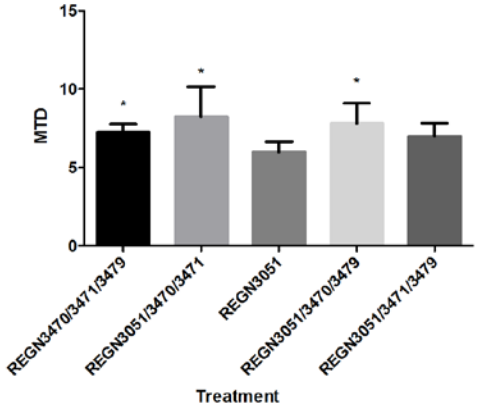
Figure 33. Clinical Scores of Treated or Mock-Treated Guinea Pigs After EBOV Exposure



Source: Figure 1, Page 23, (b) (4) 2015-001 Set#2 Study Report
REGN-EB3 is shown in red.
Abbreviations: EBOV, *Zaire ebolavirus;* REGN3051, a nonreactive control mAb. REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Guinea pigs treated with REGN-EB3, atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051, and atoltivimab (REGN3470)-maftivimab (REGN3479)-REGN3051 exhibited a significantly delayed time-to-death compared to the nonreactive antibody-treated controls (Figure 34).

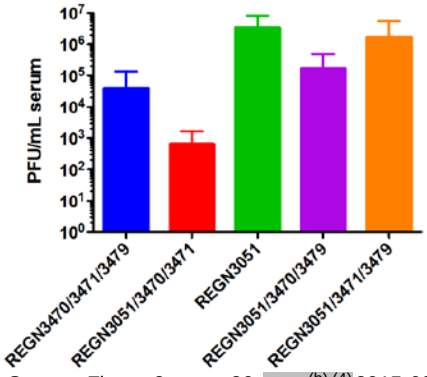
Figure 34. Mean Time-to-Death for Guinea Pigs Exposed to EBOV by the Intraperitoneal Route per Treatment Formulation



Source: Figure 2, page 24, (b) (4) 2015-001 Set#2 Study Report
*indicates samples that were statistically different from REGN3051, the control.
Abbreviations: EBOV, *Zaire ebolavirus;* REGN3051, a nonreactive control mAb. REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Viral load analysis. Viral load was analyzed by plaque assay in samples collected from all guinea pigs at Day 4 postexposure (Figure 35). REGN-EB3 (RG6) resulted in a mean viral load reduction of approximately 2 log_{10} and atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051 a mean viral load reduction of approximately 4 log_{10} compared to placebo. Atoltivimab (REGN3470)-maftivimab (REGN3479)-REGN3051 and odesivimab (REGN3471)-maftivimab (REGN3479)-REGN3051 resulted in reductions of ~1 to 2 log_{10} compared to placebo REGN3051 (Figure 35).

Figure 35. Day 4 Postexposure Serum Mean Viral Load in Guinea Pigs Challenged With EBOV by the IP Route and Treated With Different Cocktail Formulations



Source: Figure 8, page 30, (b) (4) 2015-001 Set#2 Study Report
Abbreviations: EBOV, *Zaire ebolavirus;* IP, intraperitoneal; REGN3051, a nonreactive control mAb; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

The median viral loads were 469, 363, 1.6×10^6, 0, and 300 PFU/mL in groups RG6 to RG10, respectively. The mean viral loads were 4.0×10^4, 643, 3.4×10^6, 1.7×10^5, and 1.7×10^6 PFU/mL in groups RG6 to RG10, respectively (Table 104).

Table 104. Mean and Median Day 4 Titers

Group	Treatment mAb	Mean Day-4 Titer	Median Day-4 Titer
RG6	REGN-EB3	4.0×10^4	469
RG7	REGN3470, REGN3471, REGN3051	643	363
RG8	REGN3051 (Placebo)	3.4×10^6	1.6×10^6
RG9	REGN3470, REGN3479, REGN3051	1.7×10^5	0
RG10	REGN3471, REGN3479, REGN3051	7×10^6	300

Source: DAV Analysis of Set2 Day 4 Virus Titer Data
Serum Titers Shown as PFU/mL.
Abbreviations: mAb, monoclonal antibody; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Conclusions

- Thirty Hartley guinea pigs were challenged with 1,000 PFU of EBOV-Mayinga by the IP route on study Day 0. Seventy-two hours after exposure, guinea pigs were treated with intramuscular injections of 5 mg of one of five antibody formulations (atoltivimab [REGN3470]-odesivimab [REGN3471]-maftivimab [REGN3479], atoltivimab [REGN3470]-odesivimab [REGN3471]-REGN3051, atoltivimab [REGN3470]-maftivimab [REGN3479]-REGN3051, and odesivimab [REGN3471]-maftivimab [REGN3479]-REGN3051) or a fifth irrelevant antibody control (REGN3051) and observed a minimum of twice daily for signs of disease until Day 28 postexposure.
- Each of the mAb cocktail formulations resulted in protection of 1 to 2 of 6 animals from lethal infection, with a total of 7 of the 24 treated animals surviving to the end of the study (Day 28). REGN-EB3 treatment resulted in 2 of 6 animals surviving.

- Animals treated with REGN-EB3, atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051, and atoltivimab (REGN3470)-maftivimab (REGN3479)-REGN3051 exhibited a delayed time-to-death compared to nonreactive antibody-treated controls.
- Animals treated with REGN-EB3 and odesivimab (REGN3471)-maftivimab (REGN3479)-REGN3051 showed lower clinical scores after Day 5 compared to those in the other treatment groups.
- REGN-EB3 resulted in a mean Day 4 viral load reduction of ~2 $\log_{10}$ PFU/mL and atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051 a mean Day 4 viral load reduction of ~4 $\log_{10}$ PFU/mL compared to placebo. Atoltivimab (REGN3470)-maftivimab (REGN3479)-REGN3051 and odesivimab (REGN3471)-maftivimab (REGN3479)-REGN3051 resulted in reductions of ~1 to 2 $\log_{10}$ PFU/mL compared to placebo (REGN3051).
- Of note, REGN-EB3 and atoltivimab (REGN3470)-odesivimab (REGN3471)-REGN3051 resulted in similar activity based on the clinical score, number of guinea pigs protected (two of six for both groups), delayed death, and reduction in Day 4 serum viral load. However, atoltivimab (REGN3470) and odesivimab (REGN3471), both of which signal through the FcγRIIIa pathway, contain the human Fc and therefore may not interact with guinea pig Fcγ receptors, hampering interpretation of the contribution of Fc-mediated effector activity in this model (Pascal et al. 2018).
- No resistance assessment was performed for animals that failed treatment in this study.

Overall Conclusions Based on Guinea Pig Challenge Studies

- Guinea pigs challenged with guinea pig-adapted Mayinga and treated with maftivimab (REGN3479) 1 day after challenge had the highest survival rate (Table 105).

Table 105. Summary of Data From Guinea Pig Lethal Challenge

Group	Size	Treatment	Dose	Survivors (%)	MTD (n)	Day 4 Mean Titer (PFU/mL) (n)
RG1	6	REGN3470, 1 dpi	5 mg	0 (0)	11 (6)	8.10E +02 (1)
RG2	6	REGN3471, 1 dpi	5 mg	2 (33)	9.75 (4)	3.30E +03 (1)
RG3	6	Placebo, 1 dpi	none	0 (0)	6.5 (6)	1.74E +06 (5)
RG4	6	REGN3474, 1 dpi	5 mg	0 (0)	7.5 (6)	5.66E +05 (3)
RG5	6	REGN3479, 1 dpi	5 mg	4 (67)	6.5 (2)	3.00E +06 (1)
RG6	**6**	**REGN-EB3, 3 dpi**	**5 mg**	**2 (33)**	**6.33 (4)**	**7.97E +04 (3)**
RG7	6	REGN3470-REGN3471-REGN3051, 3 dpi	5 mg	2 (33)	7.67 (4)	8.05E +02 (4)
RG8	6	REGN3051 (Placebo), 3 dpi	none	0	5 (6)	3.40E +06 (6)
RG9	6	REGN3470-REGN3479-REGN3051, 3 dpi	5 mg	1 (17)	7 (5)	5.06E +05 (2)
RG10	6	REGN3471-REGN3479-REGN3051, 3 dpi	5 mg	2 (33)	6 (4)	2.84E +06 (3)

Source: DAV Analysis
Abbreviations: dpi, days post infection; MTD, maximum tolerated dose; PFU, plaque-forming unit; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

- Although each of the mAbs individually had antiviral activity, the limited activity of atoltivimab (REGN3470) and odesivimab (REGN3471) raised concerns about breadth of activity. Clinical Virology sent these recommendations to the Applicant:
  - Virus from succumbed animals should be evaluated for the development of resistance.
  - The activity of atoltivimab (REGN3470) against VLPs derived from different outbreak strains should be determined.
- These concerns were mitigated by neutralization data for several similar EBOV strains (Section III.18.1.2.2), activity comparable to other mAb cocktails in NHP studies (below), and efficacy in human clinical trials.
- This is the only experiment that evaluated various combinations of three REGN-EB3 mAbs. Given the limitations of the model and the experimental approach, it is not clear to what extent each mAb contributes to the overall antiviral activity or if each mAb is required for the cocktail to be effective. The cocktail formulations used in this experiment dosed each mAb at one third the concentration used for the single mAb assessments.

Questions About the REGN-EB3 Cocktail That Have Not Been Addressed

1. What is the contribution of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) to the overall antiviral activity of the REGN-EB3 cocktail?
2. Are all three mAbs required for activity?
3. Do all three mAbs contribute equally to antiviral activity?
4. Is the 1:1:1 combination ratio optimal?

The limitations of the available cell culture and animal models make answering these questions challenging.

Pharmacokinetic Studies in Nonhuman Primates

Pharmacokinetic Studies in Uninfected Nonhuman Primates

Two study reports were submitted for PK studies in uninfected NHPs.

Study Title: A Single-Dose Pharmacokinetic Study of REGN3479, REGN3471 and REGN3470 Following Intravenous Administration to Rhesus Monkeys

Study Number: R3479-PM-18034

Objectives: The purpose of this study was to characterize the PK of maftivimab (REGN3479), odesivimab (REGN3471), and atoltivimab (REGN3470) after a single IV dose to uninfected rhesus monkeys.

Summary: A total of 33 animals (3 per individual mAb dose group, 6 per REGN-EB3 cocktail dose groups, approximately equal distribution of males and females) received a single IV injection of 10 mg/kg atoltivimab (REGN3470), or odesivimab (REGN3471), or maftivimab (REGN3479), or a single IV injection of 30, 100, 150, or 300 mg/kg REGN-EB3 cocktail (total dose of three mAbs given concomitantly at a 1:1:1 ratio). Blood samples for the determination of total atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) concentrations in serum were collected from all animals predose and at various time points until Day 85. The serum concentrations of the individual mAbs were assessed using a total assay format for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) by means of a validated (multiplex) electrochemiluminescent immunoassay.

Results. The mean concentration-time profiles of the individual mAbs were assessed using a total assay across all dose groups. The results for REGN-EB3 at doses of 100, 150, and 300 mg/kg are shown in Table 106. The concentration-time profiles of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) when administered as an IV bolus injection, either individually or sequentially in combination, were characterized by a brief initial distribution phase followed by a linear elimination phase throughout the duration of the study. Antibody half-life ($t_{1/2}$) was calculated during the elimination phase and ranged from approximately 10 to 14 days. Dose-proportional increases in peak concentration (C_{max}) were observed when atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) were administered individually or sequentially in combination, indicating the individual mAbs to have linear PK.

Table 106. Mean PK Parameters Following REGN-EB3 Administration in Uninfected Rhesus Macaques

		REGN-EB3 100 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
Parameter	**Unit**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**
C_{max}	µg/mL	6	770	112	14.6	6	759	110	14.5	6	779	109	14
C_{max}/Dose	(µg/mL)/(mg/kg)	6	23.1	3.38	14.6	6	22.8	3.3	14.5	6	23.4	3.28	14
t_{max}	h	6	0.083	0	0	6	0.083	0	0	6	0.083	0	0
AUC_{inf}	day•(µg/mL)	6	7760	918	11.8	6	6360	846	13.3	6	5080	966	19
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	6	233	27.6	11.8	6	191	25.4	13.3	6	153	29	19
$t_{1/2}$	day	6	14.2	1.27	8.96	6	11.4	1.42	12.5	6	11.6	1.75	15.1
CL	mL/day/kg	6	4.34	0.516	11.9	6	5.33	0.796	14.9	6	6.74	1.22	18.1
V_{ss}	mL/kg	6	83.5	7.6	9.09	6	79.5	5.31	6.68	6	89.1	6.22	6.99
		REGN-EB3 150 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
Parameter	**Unit**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**
C_{max}	µg/mL	6	1100	104	9.41	5	1160	70.5	6.07	6	1150	185	16.1
C_{max}/Dose	(µg/mL)/(mg/kg)	6	22.1	2.08	9.41	5	23.2	1.41	6.07	6	23	3.71	16.1
t_{max}	h	6	0.083	0	0	5	0.866	1.75	202	6	0.083	0	0
AUC_{inf}	day•(µg/mL)	5	10600	2120	20.1	4	9840	472	4.79	5	6700	340	5.08
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	5	211	42.5	20.1	4	197	9.43	4.79	5	134	6.8	5.08
$t_{1/2}$	day	5	13.5	2.91	21.5	4	12.5	1.06	8.46	5	10.9	2.65	24.4
CL	mL/day/kg	5	4.94	1.28	26	4	5.09	0.236	4.64	5	7.48	0.383	5.12
V_{ss}	mL/kg	5	87.3	11.2	12.8	4	81.5	11.3	13.9	5	96.7	23.3	24
		REGN-EB3 300 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
Parameter	**Unit**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**
C_{max}	µg/mL	6	1960	126	6.46	6	1940	150	7.73	6	1950	171	8.76
C_{max}/Dose	(µg/mL)/(mg/kg)	6	19.6	1.26	6.46	6	19.4	1.5	7.73	6	19.5	1.71	8.76
t_{max}	h	6	1.39	2.02	146	6	0.736	1.6	217	6	1.39	2.02	146
AUC_{inf}	day•(µg/mL)	6	21300	3080	14.4	6	16300	3330	20.4	6	11100	2800	25.1
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	6	213	30.8	14.4	6	163	33.3	20.4	6	111	28	25.1
$t_{1/2}$	day	6	14.2	1.67	11.8	6	11.9	1.69	14.3	6	11.7	1.86	15.9
CL	mL/day/kg	6	4.77	0.67	14.1	6	6.35	1.27	20	6	9.45	2.32	24.6
V_{ss}	mL/kg	6	96.8	7.46	7.7	6	99.9	7.2	7.2	6	127	12	9.43

Source: Modified from Table 2, page 189, R3479-PM-18034 Study Report
*Concentration values considered to be ADA-impacted were excluded from data analysis.
Abbreviations: ADA, antidrug antibodies; AUC, area under the concentration-time curve; AUC_{inf}, AUC from time zero extrapolated to infinity; CL, total body clearance; C_{max}, peak concentration; CV, coefficient of variation; h, hours; IV, intravenous; N, number of animals; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; SD, standard deviation; $t_{1/2}$, elimination half-life; t_{max}, time to C_{max}; V_{ss}, volume of distribution at steady state

Conclusions

1. Overall, no meaningful differences in PK parameters were observed among atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) dosed individually or as REGN-EB3. This indicates the absence of PK interactions among the three individual mAbs when dosed in combination.
2. Dose-proportional increases in C_{max} and AUC_{inf} indicated linear kinetics of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) when dosed in combination. The comparable dose-normalized C_{max} and AUC values for all mAbs, as well as the similar concentration-time profiles, provided evidence of linear kinetics for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) when dosed individually.

Considerations for PMR/PMC: None

Study Title: REGN3479, REGN3471, and REGN3470: A Single-Dose Intravenous Infusion Pharmacokinetics Study in Cynomolgus Monkeys

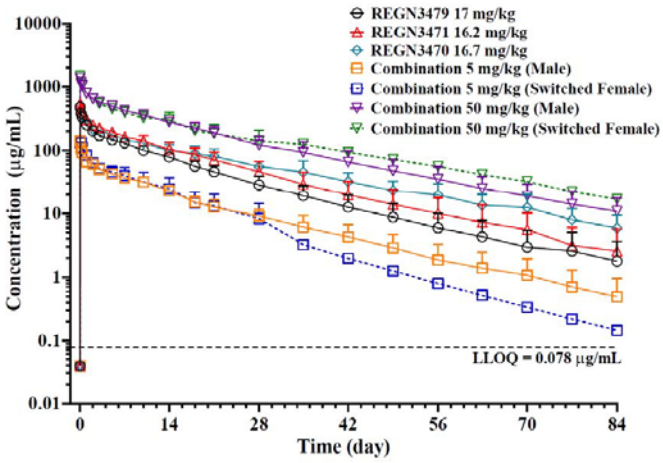
Study Number: REGN3479-PK-15086

Objectives: The objective of this study was to determine the PK characteristics of maftivimab (REGN3479), odesivimab (REGN3471) and atoltivimab (REGN3470), when given individually or in combination as a single 30-minute IV infusion to uninfected cynomolgus monkeys.

Results

The PK of atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), and REGN-EB3 was evaluated following a single IV infusion of ~17 mg/kg of each of the individual mAbs or 5 or 50 mg/kg of REGN-EB3 (total dose of the three mAbs given in combination at a 1:1:1 ratio) to male and female cynomolgus monkeys. The concentration-time profiles were characterized by an initial brief distribution phase followed by a linear beta-elimination phase throughout the 84-day study duration (Figure 36). Peak individual REGN-EB3 mAb concentrations (C_{max}) and exposures (AUC_{inf}) to atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) increased in a dose-proportional manner when dosed individually or in combination, indicating linear kinetics. Clearance values were dose-independent, the volume of distribution (V_{ss}) was comparable across dose groups, and terminal elimination half-lives ($t_{1/2}$) ranged from approximately 8.3 to 16.6 days. C_{max} values greater than 300 µg/mL were observed for the 50 mg/kg combination and for each of the mAbs when dosed individually at 16.2 to 17 mg/kg. The Applicant noted that theoretically, these C_{max} values (which equate to $>10^{15}$ antibodies/mL) are in the range required to saturate viral titers greater than 1×10^{10} PFU/mL. However, Clinical Virology does not agree given that the EBOV particle-to-PFU ratio is on the order of 10^2 to 10^4, there are hundreds of GP molecules/virus particle, and sGP would have a greater impact on odesivimab (REGN3471).

Figure 36. Mean (+SD) Total Anti-Ebola Antibody Concentrations in Serum vs. Time Following a Single Intravenous Infusion of Maftivimab (REGN3479), Odesivimab (REGN3471), and Atoltivimab (REGN3470) Individually or in Combination in Cynomolgus Monkeys



LLOQ = Lower limit of quantitation
Note: Predose BLQ values are imputed as ½ LLOQ (0.039 µg/mL).
Combination denotes the total dose of REGN3479, REGN3471, and REGN3470 combined in a 1:1:1 ratio and administered as a single IV infusion. For the combination groups, the 5 mg/kg switched females are from the 50 mg/kg group and the 50 mg/kg switched females are from the 5 mg/kg group (Section 2.2.2). n = 3 animals/sex/group.
Concentrations likely impacted by ADA (two females dosed with 16.2 mg/kg REGN3471 with data excluded starting on Day 19, one female given a combined dose of 5 mg/kg with data excluded starting on Day 36, and one female given a combined dose of 50 mg/kg with data excluded starting on Day 36) were excluded from mean calculations (Table 15).

Source: Figure 1, page 347, REGN3479-PK-15086 Study Report
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Conclusions

1. No meaningful differences in PK parameters were observed between maftivimab (REGN3479), odesivimab (REGN3471), and atoltivimab (REGN3470) when dosed individually or in combination, indicating that there were no PK interactions between the three individual mAbs when dosed in combination.
2. Dose-proportional increases in C_{max} and AUC_{inf} indicate linear kinetics of maftivimab (REGN3479), odesivimab (REGN3471), and atoltivimab (REGN3470) when dosed in combination, and the comparable dose-normalized C_{max} and AUC_{inf} values for all mAbs provide evidence of linear kinetics for maftivimab (REGN3479), odesivimab (REGN3471), and atoltivimab (REGN3470) when dosed individually.
3. C_{max} values >300 µg/mL were observed for the 50 mg/kg combination and for each of the mAbs when dosed individually at 16.2 to 17 mg/kg. Theoretically, these C_{max} values (equate to $>10^{15}$ antibodies/mL) are in the range required to saturate viral titers greater than 1×10^{10} PFU/mL.

Considerations for PMR/PMC: None

Pharmacokinetic Studies in Nonhuman Primates Infected with EBOV

One study report was submitted for PK Studies in NHPs infected with EBOV.

Study Title: A Pharmacokinetic/Pharmacodynamic Assessment of REGN3470-3471-3479 in Rhesus Macaque Monkeys Infected with Ebola Virus

Study Number: (b) (4) 2018-008-R3479-PM-18140

Objectives: The purpose of this study was to determine the PK/PD relationship between REGN-EB3 mAb concentrations and viral titers in a rhesus macaque model of EBOV infection.

Study design: In this blinded and randomized study conducted at (b) (4), 23 animals (4 or 5 per dose group with an approximately equal distribution of males and females) received a single IV injection of 0 (placebo), 30, 100, 150, or 300 mg/kg REGN-EB3 (total dose of the three mAbs given concomitantly at a 1:1:1 ratio) 5 days after being infected (on Day 0) by IM injection with a target of 1,000 PFU of EBOV (EBOV Kikwit with 95% 7U; Lot No. 201209171); animals were not stratified by viral titer. Survival was assessed until the end of study and included established clinical score criteria for euthanasia. Animals that did not succumb to EBOV were sacrificed from days 47 to 49. Viral titers in serum were determined by quantitative RT-PCR (qRT-PCR) at days 5 (Day 5, pretreatment), 6, 8, 12, 19, 26, 33, 40, and 47 to 49; additionally, due to a lack of serum samples for four animals on Day 5, viral titers in plasma were determined for all animals on Day 5 (pretreatment) only. Pretreatment serum sGP levels were determined on Day 5 for all dose groups; for the placebo group, postdose blood samples were also drawn at the same time points as indicated above for the viral titers. Blood samples for the determination of total atoltivimab (REGN3470), total odesivimab (REGN3471), and total maftivimab (REGN3479) concentrations in serum were collected from all animals at the same timepoints as those for determination of viral titers.

Results

PK. The mean concentration-time profiles of the individual mAbs assessed using a total assay across all dose groups was conducted. The results for REGN-EB3 doses of 100, 150, and 300 mg/kg are shown in Table 107. The concentration-time profiles of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) when administered as an IV bolus injection, either individually or sequentially in combination, were characterized by a brief initial distribution phase followed by a linear elimination phase throughout the duration of the study. Antibody half-life ($t_{1/2}$) during the elimination phase ranged from 10 to 14 days. Dose-proportional increases in peak concentration (C_{max}) were observed when atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) were administered individually or sequentially in combination, indicating the individual mAbs to have linear PK.

Table 107. Mean PK Parameters Following REGN-EB3 Administration in EBOV-Infected Rhesus Macaques

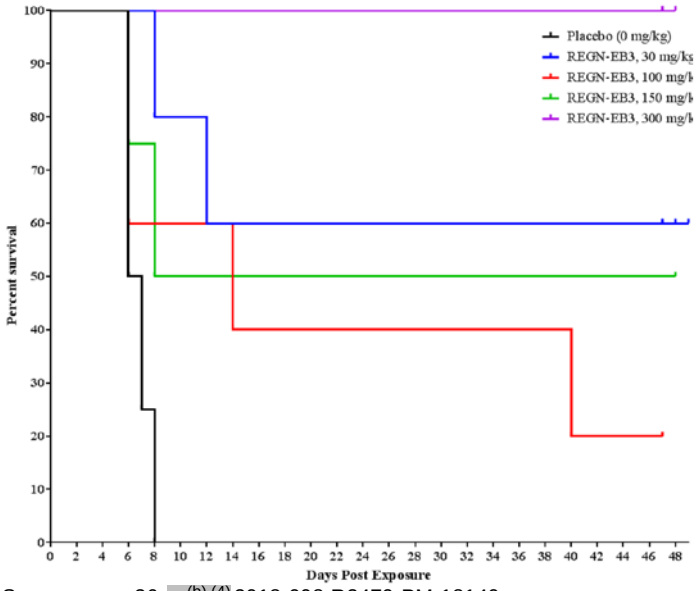
		REGN-EB3 100 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
Parameter	**Unit**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**
C_{max}	µg/mL	5	801	131	16.4	5	734	150	20.5	5	794	125	15.7
C_{max}/Dose	(µg/mL)/(mg/kg)	5	24	3.93	16.4	5	22	4.51	20.5	5	23.9	3.74	15.7
t_{max}	h	5	0.0833	0	0	5	0.0833	0	0	5	0.0833	0	0
AUC_{inf}	day•(µg/mL)	5	3,110	1,670	53.8	5	1,860	1,420	76.4	5	2,150	942	43.8
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	5	93.3	50.2	53.8	5	56	42.8	76.4	5	64.5	28.3	43.8
$t_{1/2}$	day	5	4.17	2.88	69.1	5	2.01	1.89	94.2	5	2.9	2.05	70.8
CL	mL/day/kg	5	16	13.8	86.4	5	36.6	33	90.2	5	20.1	13.7	68.2
V_{ss}	mL/kg	5	54	13.2	24.4	5	43.6	22	50.4	5	52.5	20.7	39.4
		REGN-EB3 150 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
Parameter	**Unit**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**
C_{max}	µg/mL	4	1120	448	40	4	1030	410	39.7	4	1070	378	35.3
C_{max}/Dose	(µg/mL)/(mg/kg)	4	22.4	8.96	40	4	20.6	8.2	39.7	4	21.4	7.55	35.3
t_{max}	h	4	0.0833	0	0	4	0.0833	0	0	4	0.0833	0	0
AUC_{inf}	day•(µg/mL)	4	4,400	2,420	55.1	4	3,410	2,260	66.2	4	3,190	1,600	50.1
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	4	88.1	48.5	55.1	4	68.2	45.1	66.2	4	63.8	32	50.1
$t_{1/2}$	day	4	4.01	3.29	82.1	4	3.53	3.72	105	4	3.37	3.02	89.5
CL	mL/day/kg	4	14.7	8.06	55	4	24.4	21.9	89.9	4	19.6	10.8	55.1
V_{ss}	mL/kg	4	57.8	24.6	42.6	4	60.8	31.5	51.9	4	57.1	22.5	39.3
		REGN-EB3 300 mg/kg (1:1:1)											
		REGN3470				REGN3471				REGN3479			
Parameter	**Unit**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**	**N**	**Mean**	**SD**	**CV%**
C_{max}	µg/mL	5	2,050	478	23.3	5	2,040	452	22.2	5	2,030	466	23
C_{max}/Dose	(µg/mL)/(mg/kg)	5	20.5	4.78	23.3	5	20.4	4.52	22.2	5	20.3	4.66	23
t_{max}	h	5	0.0833	0	0	5	0.0833	0	0	5	0.0833	0	0
AUC_{inf}	day•(µg/mL)	5	18,500	3,690	20	5	15,000	2,340	15.6	5	10,700	1,940	18.2
AUC_{inf}/Dose	day•(µg/mL)/(mg/kg)	5	185	36.9	20	5	150	23.4	15.6	5	107	19.4	18.2
$t_{1/2}$	day	5	9.54	3.19	33.5	5	7.93	1.71	21.5	5	7.01	1.36	19.3
CL	mL/day/kg	5	5.64	1.4	24.9	5	6.77	1.03	15.2	5	9.61	1.75	18.2
V_{ss}	mL/kg	5	73.6	17.3	23.5	5	72.8	15.2	20.9	5	82.9	21.4	25.8

Source: Modified from Table 2, page 189, R3479-PM-18034 Study Report.
*Concentration values considered to be affected by ADA were excluded from the data analysis.
Abbreviations: ADA, antidrug antibodies; AUC, area under the concentration-time curve; AUC_{inf}, AUC from time zero extrapolated to infinity; CL, total body clearance; C_{max}, peak concentration; CV, coefficient of variation; h, hours; IV, intravenous; N, number of animals; SD, standard deviation; $t_{1/2}$, elimination half-life; t_{max}, time to C_{max}; V_{ss}, volume of distribution at steady state. REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Survival. A total of 23 animals received a single IV injection of 0 (placebo; n=4), 30 (n=5), 100 (n=5), 150 (n=4), or 300 (n=5) mg/kg REGN-EB3 5 days postchallenge on Day 0 by IM injection with a target of 1,000 PFU of EBOV Kikwit. The EBOV exposure dose calculated by plaque assay using an aliquot removed from the exposure material prior to animal exposure was 875 PFU. The survival results are presented below and in Figure 37.

1. **Group 1:** All placebo-treated animals were euthanized due to moribundity: two on Day 6, one on Day 7, and one on Day 8. N=4; 100% mortality.
2. **Group 2:** In the 30 mg/kg treatment group: one animal was euthanized due to moribundity on Day 8 and one was found dead on Day 12; the remaining three survived until the scheduled end of study. N=5; 40% mortality.
3. **Group 3:** In the 100 mg/kg treatment group: on Day 6 one animal was euthanized due to moribundity and one was found dead, one animal was euthanized due to moribundity on Day 14, one animal was found dead on Day 40; the remaining animal survived until the scheduled end of study. N=5; 80% mortality.
4. **Group 4:** In the 150 mg/kg treatment group: one animal was euthanized due to moribundity on Day 6 and one was found dead on Day 8; the remaining two animals survived until the scheduled end of study. N=4; 50% mortality.
5. **Group 5:** All five animals in the 300 mg/kg treatment group survived until the scheduled end of study. N=5; 0% mortality.

Figure 37. Survival in NHPs Exposed to EBOV and Subsequently Treated With REGN-EB3 or Placebo



Source: page 36, (b) (4) 2018-008-R3479-PM-18140
Abbreviations: EBOV, *Zaire ebolavirus;* NHPs, nonhuman primates

PK in Infected NHPs

- In the presence of EBOV infection, the Applicant reported that the PK profiles of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) following a single IV administration of REGN-EB3 (30, 100, 150 or 300 mg/kg) to EBOV-infected rhesus macaques indicated target-mediated clearance with high viral load having an effect on PK, especially at the lower REGN-EB3 dose levels. Following a 30 mg/kg dose of the REGN-EB3 cocktail, the concentration-time profiles for atoltivimab

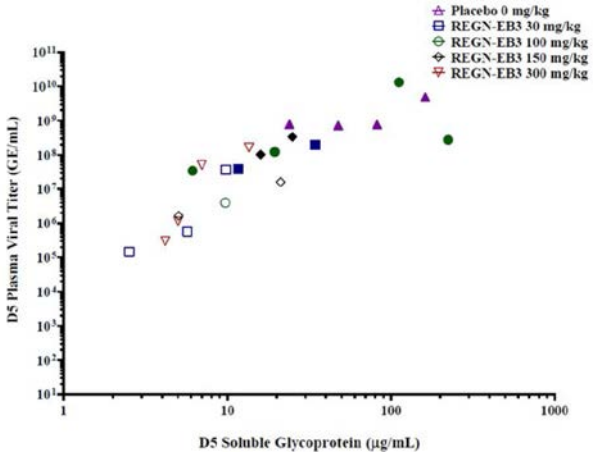
(REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) in animals with a high viral load were characterized by a brief distribution phase followed by a steep elimination phase relative to animals with a low viral load. This is likely a result of target-mediated clearance. Following administration of the 100 mg/kg and 150 mg/kg doses of the REGN-EB3 cocktail, the beta phase is more distinct and the presumptive target-mediated phase only apparent in animals with the highest Day-5 viral titers. Following administration of the 300 mg/kg dose of the REGN-EB3 cocktail, a prolonged beta-elimination phase predominates throughout the 47-day study duration.

- Consistent with the observed nonlinear kinetics, clearance was dose-dependent. Based on the 300 mg/kg dose level, which showed a prolonged beta-elimination phase, the mean beta half-life ($t_{1/2}$) was estimated to be approximately 9.5, 7.9, and 7.0 days for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479), respectively.
- The Applicant reported a subtle indication of enhanced clearance of odesivimab (REGN3471) relative to atoltivimab (REGN3470) and maftivimab (REGN3479) in animals with the highest sGP concentrations and highest viral titers; the dose-normalized AUC was slightly lower for odesivimab (REGN3471) in the 30 mg/kg and 100 mg/kg groups. The dose-normalized C_{max} values were similar for the individual mAbs and across dose groups.
- In summary, a high viral load and high sGP level at baseline on Day 5 (pretreatment) was associated with a lower survival rate of treated animals. All animals in the 300 mg/kg dose group survived; survival in the 100 and 150 mg/kg dose groups was lower in this study as compared to previous studies with REGN-EB3 in infected rhesus macaque monkeys. The small number of animals per group in combination with the skewed distribution of viral load and sGP level across groups made problematic drawing conclusions on efficacy across the dose groups assessed. Therefore, it is difficult to accurately assess whether the 300 mg/kg dose has a survival benefit over the 150 mg/kg dose.
- Disease appeared to impact PK of REGN-EB3; while the concentration-time profiles are somewhat limited due to the high mortality in this study, there is some evidence to suggest target-mediated clearance in infected animals with higher viral titers. As described above, there was a subtle trend of preferential clearance of odesivimab (REGN3471) in the presence of high viral load and sGP, though the kinetics of the three individual mAbs were not markedly different in the majority of animals.

Day 5 sGP and Viral Titers

- The relationship between Day 5 (pretreatment) viral titers, Day 5 (pretreatment) sGP concentrations, and survival outcome in treated animals was assessed.
- The Applicant reported that in all animals, the Day 5 viral titers trended with the Day 5 sGP levels, with higher viral titers typically associated with higher sGP levels.
- Treated animals that succumbed to infection tended to have higher Day 5 viral titers and sGP levels relative to treated animals that survived infection (Figure 38).

Figure 38. Individual Day 5 Plasma Viral Titers vs. Day 5 sGP Concentrations Prior to Treatment With REGN-EB3 or Placebo in NHPs Infected With EBOV



Source: Figure 2, page 1625, Study R3479-PM-18140
Notes: REGN3470-3471-3479 cocktail is also known as REGN-EB3 in this document. N=5 animals for the 30 and 100 mg/kg dose groups; N=4 animals for the control, 150, and 300 mg/kg dose groups, one value in the 300 mg/kg group was not plotted due to a nonreportable result. Solid symbols denote death of an animal.
Abbreviations: EBOV, *Zaire ebolavirus;* NHPs, nonhuman primates; sGP, soluble glycoproteins

- Compared to the other dose groups, animals who received 300 mg/kg generally had lower Day 5 sGP concentrations and pretreatment baseline viral titers.
- Therefore, the Applicant stated that it was difficult to accurately assess whether the 300 mg/kg dose had a survival benefit over the 150 mg/kg dose.

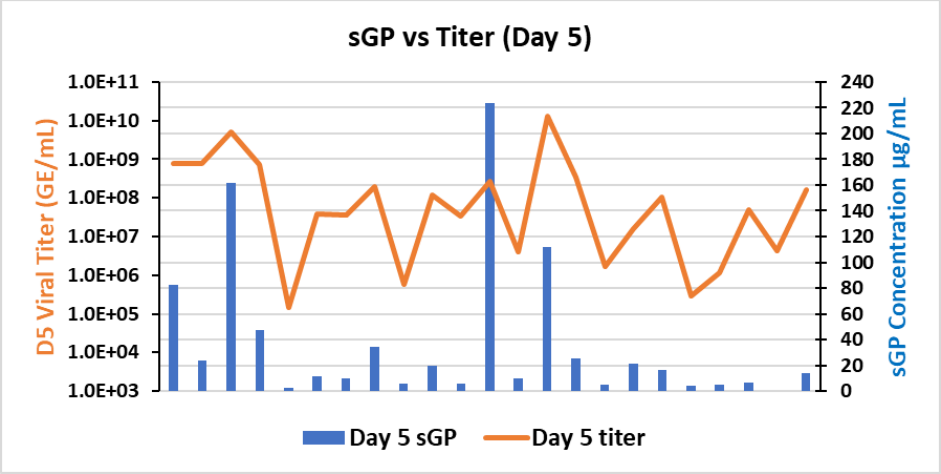
Clinical Virology has communicated a concern to Regeneron that one of the mAbs, odesivimab (REGN3471), which binds to sGP as well as to GP on the EBOV virion, might be bound by sGP in serum, thus preventing it from contributing to efficacy as part of the three-mAb cocktail. This concern is based on two assumptions:

- That all three of the mAbs in the REGN-EB3 cocktail must be present in excess of their corresponding epitope for antiviral activity.
- sGP is in 100- to 1,000-fold excess of EBOV in the serum, which would reduce the amount of odesivimab (REGN3471) available to contribute to antiviral activity and efficacy.

Clinical Virology performed an independent assessment of odesivimab (REGN3471) PK, time to death (D), and Day 5 sGP levels and viral titers among the dose groups to assess whether high Day 5 viral titers and sGP levels are correlated with death and whether high sGP levels affected the PK of odesivimab (REGN3471).

Comparing Day 5 (pretreatment) sGP and viral titers, there was a general trend indicating that high sGP levels were associated with high Day 5 viral titers (Figure 39). All animals that had Day 5 sGP levels >20 µg/mL had Day 5 viral titers $>10^8$ GE/mL, with the exception of one NHP that had a Day 5 sGP level of 21.2 µg/mL and a Day 5 viral titer of 1.6×10^7 GE/mL (Figure 39). Three additional NHPs had Day 5 viral titers $>10^8$ GE/mL and Day 5 sGP levels of 19.5, 16, and 13.6 µg/mL (Figure 39). This trend supports the concern that NHPs and potentially human patients with high viral titers at baseline may have excessive sGP levels that interfere with odesivimab (REGN3471).

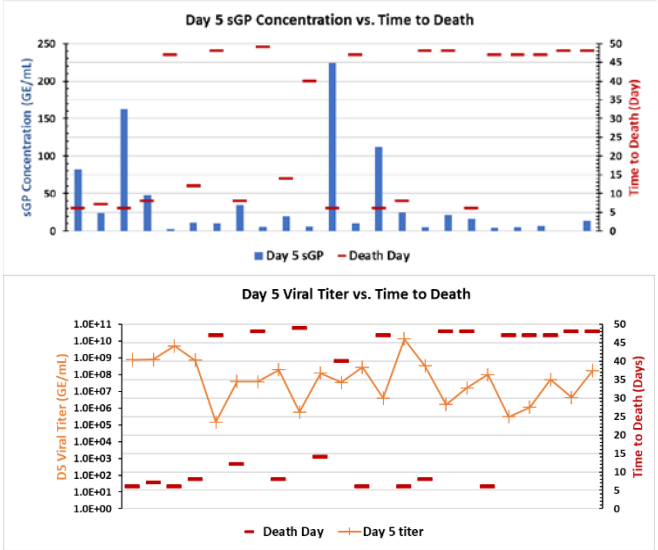
Figure 39. Day 5 sGP Concentration Compared to Viral Titer at Day 5



Source: DAV Analysis
X-axis contains n=23 NHPs from all dose groups
Abbreviation: sGP, secreted glycoprotein

To determine if the sGP concentration and/or viral titer at Day 5 (pretreatment) affected the overall time to death, the sGP concentrations at Day 5 and viral titers at Day 5 were compared to day of death for all NHPs. Overall, higher Day 5 sGP concentrations and viral titers were associated with more rapid death; however, there were outliers that did not correlate with these trends (Figure 40).

Figure 40. Day 5 sGP Concentration Compared to Viral Titer at Day 5 and Time to Death

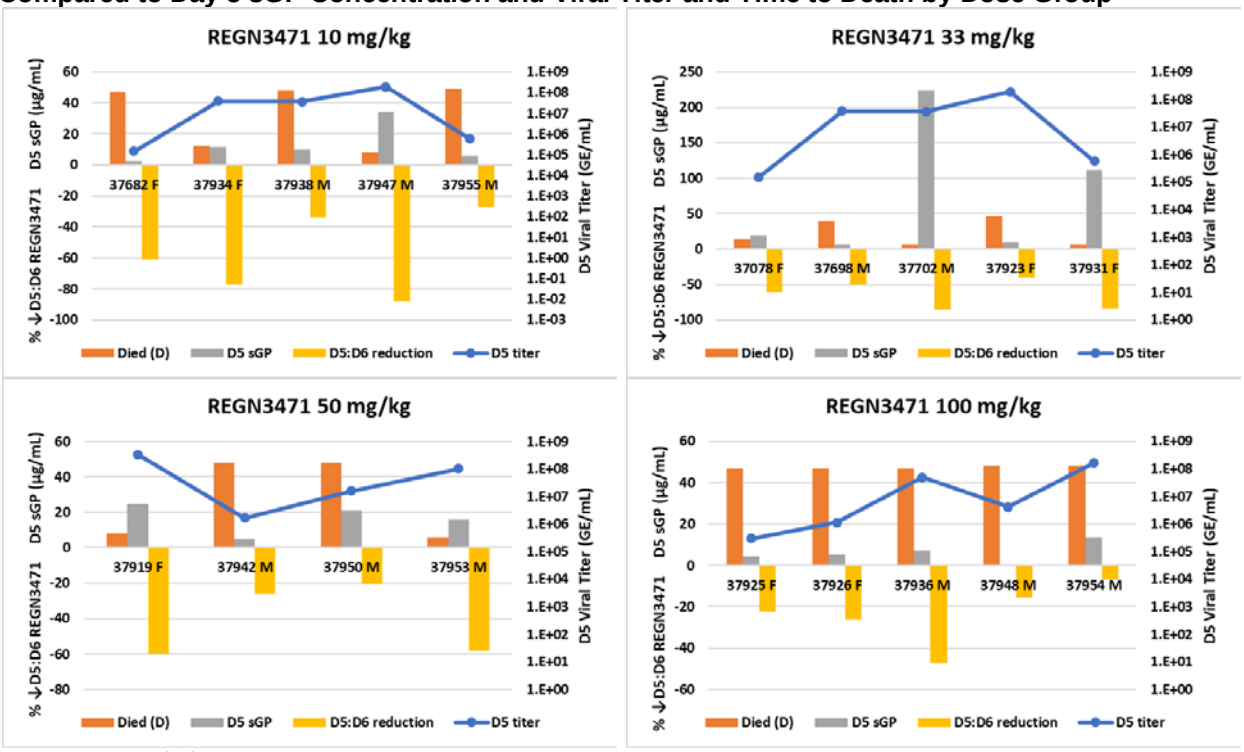


Source: DAV Analysis
X-axis contains n=23 NHPs from all dose groups
Abbreviation: sGP, secreted glycoprotein

Analysis of the relationships among Day 5 (pretreatment) viral titers, Day 5 (pretreatment) sGP concentrations, and survival outcome in treated animals showed a positive correlation between the baseline sGP concentration and the baseline viral titer. Treated animals that succumbed to infection tended to have higher Day 5 plasma viral titers (3.4×10^{7} to 1.3×10^{10} GE/mL) relative to treated animals that survived infection (1.5×10^{5} to 1.6×10^{8} GE/mL).

To determine if the PK of odesivimab (REGN3471) was impacted by higher Day 5 viral titers and sGP concentrations, the percentage reduction in odesivimab (REGN3471) concentration from Day 5 to Day 6 was compared to the sGP concentration, Day 5 viral titer, and time to death by dose group (Figure 41).

Figure 41. Percentage Reduction in Odesivimab (REGN3471) Concentration From Day 5 to Day 6 Compared to Day 5 sGP Concentration and Viral Titer and Time to Death by Dose Group



Source: DAV Analysis
Abbreviation: D5, Day 5; D5:D6 Reduction, percentage reduction in REGN3471 concentration from 5 min after administration on Day 5 to Day 6 in infected NHPs; REGN3471, odesivimab; sGP, secreted glycoprotein

In general, higher Day 5 sGP and viral titers correlated with greater reductions in odesivimab (REGN3471) concentrations from 5 min after administration on Day 5 to Day 6 in infected NHPs that received 10 and 33 mg/kg of odesivimab (REGN3471) (30 mg/kg and 100 mg/kg of REGN-EB3, respectively) (Figure 41). However, this trend did not hold for the higher dose groups (Figure 41).

In summary, the data indicate that high viral load had an impact on PK, especially at the lower REGN-EB3 dose levels. There was a subtle trend of preferential clearance of odesivimab (REGN3471) in the presence of a high viral load and high sGP concentration, though the kinetics of the three individual mAbs were not markedly different in the majority of animals. A high viral load and a high sGP concentration at baseline on Day 5 (pretreatment) were associated with a lower survival rate of treated animals. A greater number of animals survived following treatment with REGN-EB3 than placebo; all animals dosed with 300 mg/kg REGN-EB3 survived. The small number of animals per group in combination with the skewed distribution of viral load and sGP concentration across groups made it difficult to draw conclusions on efficacy across the dose groups assessed.

The assessment of resistance for NHPs in this study is discussed in Section III.18.1.2.5).

Conclusions

1. High viral titers and sGP concentrations at Day 5 influenced time to death and indicate that higher doses of REGN-EB3 might provide protection. However, in the higher-dose group tested in this study, none of the animals had high sGP concentrations at Day 5. It is not clear why Day 5 sGP concentrations and viral titers are not more closely aligned.
2. High viral load had an impact on PK, especially at the lower REGN-EB3 dose levels. There was a subtle trend of preferential clearance of odesivimab (REGN3471) in the presence of a high viral load and high sGP concentration, although the kinetics of the three individual mAbs were not markedly different in the majority of animals.
3. High viral load and high sGP concentration at baseline on Day 5 (pretreatment) were associated with a lower survival rate of treated animals. A greater number of animals survived following treatment with than placebo; all animals dosed with 300 mg/kg REGN-EB3 survived.
4. The small number of animals per group in combination with the skewed distribution of viral load and sGP across groups makes it difficult to draw conclusions on efficacy across the dose groups assessed.

Considerations for PMR/PMC

1. High viral load and high sGP concentration at baseline on Day 5 (pretreatment) were associated with a lower survival rate of treated animals, providing evidence that higher doses may be required to treat NHPs and humans with high baseline viral titers and sGP concentrations at the time of treatment initiation.
2. The NHP model is too variable for assessing higher doses. Dose-optimization studies should be performed in clinical trials during outbreaks, in which subjects with high baseline viral loads (CtNP <22) should receive doses of 300 mg REGN-EB3.

Antiviral Activity Studies in Nonhuman Primates

Studies in NHPs were conducted to establish that REGN-EB3 has antiviral activity and to perform the initial dose assessments, given that REGN-EB3 development was initially undertaken under the assumption of an Animal Rule approval. The studies described in this section focus on assessments of survival in the lethal EBOV challenge models, quantification of EBOV by plaque assay, quantification of EBOV RNA by RT-PCR, and quantification of sGP concentrations at the time of treatment initiation, if sGP was measured. These studies involved determination of other parameters of infection such as clinical symptoms, weight loss, body temperature, and clinical chemistry values; however, while these parameters were reviewed in the context of the overall study, they did not provide any additional significant insights into the antiviral activity (defined by survival and reduction in viral load) and so they are not discussed.

Study Title: Single Dose of a Monoclonal Antibody Cocktail at Varying Concentrations to Reverse Ebola Virus Disease in Rhesus Macaques

Study Number: AP-14-017-IX-X

Protocol: AP-14-017 IX and AP-14-017 X

Note: The Applicant stated that the studies were combined to achieve statistical significance; a sample size of nine treated animals per treatment group and four control animals provided adequate (85%) power to detect a difference in survival rates assuming

at least 78% survival in the treatment groups versus <1% survival in the control group at a one-tailed alpha of 0.05 (Fisher's exact test). The <1% survival rate in the control group is not consistent with those in previous blinded studies.

Objectives: AP-14-017 IX and AP-14-017 X were randomized, blinded studies to assess the antiviral activity of REGN-EB3 at doses of 150, 100, 50, or 10 mg/kg delivered IV starting at Day 5 (5 days after infection) in rhesus macaques challenged with 1,000 PFU of EBOV (EBOV Kikwit strain R4415; 7U).

Institute that conducted the study: U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)

Animals: Forty research-naïve rhesus macaques of Asian origin, 20 males and 20 females, were included in the study. The macaques had a starting age range of 3 to 6 years and an initial body weight range of 3.7 to 10.0 kg on Day 0 (females weighed 4.3 to 6.3 kg and males weighed 3.7 to 10.0 kg).

Challenge strain: The EBOV Kikwit strain (lot #R4415; 7U 95%; AY354458) was derived from a 65-year-old female during an outbreak that occurred in 1995 in the DRC (formerly Zaire). The patient exhibited disease, was hospitalized, and died. Of note, GP substitution T544I is a tissue-culture adaptation known to arise in certain cell lines (Hoffmann et al. 2017; Ruedas et al. 2017; Ueda et al. 2017); however, the presence of this polymorphism was not addressed in the study report. It appears that the T544I substitution may be associated with cell tropism or an unknown selective pressure that is unrelated to treatment with REGN-EB3.

Euthanasia Criteria

Primary Euthanasia Criteria

0=alert, responsive, normal activity, free of disease signs or exhibits only resolved/resolving disease signs

1=slightly diminished general activity, subdued but responds normally to external stimuli

2=withdrawn, may have head down, fetal posture, hunched, reduced response to external stimuli

3=prostrate but able to rise if stimulated, moderate to dramatically reduced to response to external stimuli

4=persistently prostrate, severely or completely unresponsive, may have signs of respiratory distress

If primary euthanasia criteria=1 to 3, increase frequency of observations IAW protocol and notify Study Director

If primary euthanasia criteria=3, complete form "Exposed NHP Euthanasia Criteria" IAW protocol

If primary euthanasia criteria=4, then criteria have been met and euthanasia is required

If primary euthanasia criteria=3, evaluate temperature and blood chemistry via secondary euthanasia criteria

Secondary Euthanasia Criteria

1. Rectal temperature ≤34°C; if yes, euthanize animal; if no, analyze chemistry values
2. BUN ≥68 mg/dL
3. GGT ≥391 U/dL

4. CA ≤6.8 mg/dL
5. CRE ≥2.8 mg/dL
6. Telemetry temperature ≥4°C below baseline

Experimental Design: Study personnel were blinded to avoid bias pertaining to the identity of the study groups. A total of 40 rhesus macaques (20 from each study) were divided into five treatment groups. On Day 0, all animals were exposed by the IM route to EBOV, (EBOV Kikwit R4415; 7U), with a target dose of 1,000 PFU. Five days after infection, all animals were treated with a single IV dose of saline or REGN-EB3 at 150, 100, 50, or 10 mg/kg.

Virologic Assessments

1. **Mortality:** The primary endpoint for these studies was survival ≥28 days postchallenge. Mortality was defined as the day on which an animal is FDIC or the day an animal was euthanized following the criteria used by the study institution.
2. **Plaque assay for serum EBOV RNA analysis: All animals (both studies) had** viremia assessed on days 3, 5, 8, 11, 15, and prior to unscheduled euthanasia, whenever possible. In addition, for AP-14-017 X, viremia was also assessed on days 18, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, and 119 following EBOV exposure.
3. **Cerebrospinal fluid plaque assay for EBOV RNA analysis:** Cerebrospinal fluid was collected from AP-14-017 X on days 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, and 119 from all surviving animals to check for viral persistence by plaque assay, whenever possible. No plaques were detected for any of the animals at the intervals examined when using cerebrospinal fluid in this assay.
4. **qRT-PCR for circulating EBOV genomes:** All animals (both studies) had serum samples collected to assess circulating EBOV genomes on days 0 to 11, 15, 18, 21, 28, and 35; in addition, for AP-14-017 X, samples were also assessed on days 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, and 119 following EBOV exposure. The lower limit of quantitation (LLOQ) was 4.9031 $\log_{10}$ GE/mL; values below the LLOQ were not plotted.
5. **Resistance monitoring:** The protocol indicated that samples were to be assessed for viral genome sequencing. The study investigators reported that it remains their intention to conduct this assay in the future; however, the assay has been postponed indefinitely until the appropriate methods and standard operating procedures (SOPs) can be obtained. There is no timeframe established for this assay to be completed and it was not included in this study report. When the data from this assay are available, a separate report will be completed.

Virology Assays

1. **qRT-PCR:** Inactivated samples were extracted and eluted with AVE Buffer using a QIAamp Viral RNA Mini Kit. All samples were used in quantitative RT-PCR on an ABI 7500 Fast Dx. The RT-PCR reaction used SuperScript II One-Step RT-PCR System (Invitrogen) with additional $MgSO_4$ added to a final concentration of 3.0mM. The sequence of the primer and probe for the EBOV GP gene were:
2. **Forward primer (1µM):** 5′-TTT TCA ATC CTC AAC CGT AAG GC-3′
3. **Reverse primer (1µM):** 5′-CAG TCC GGT CCC AGA ATG TG-3′
4. **Probe (0.1µM):** 6FAM-CAT GTG CCG CCC CAT CGC TGC–TAMRA

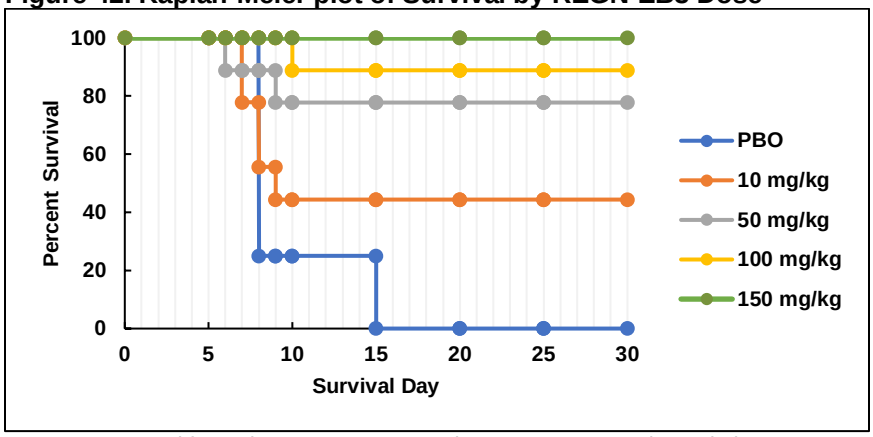
5. The genomic equivalents were determined using a standard curve of synthetic RNA of known concentration. The LLOQ was 4.9031 $\log_{10}$ GE/mL; values below the LLOQ were not plotted.
6. **Plaque Assay:** The limit of detection (LOD) for the plaque assay as performed was set at 16.5 PFU/mL, the LLOQ was set at 500 PFU/mL, and the upper limit of quantitation was set at 7.5×10^8 PFU/mL.
7. **Viral Genome Sequencing:** The study report indicated that (b) (4) will utilize whole-blood specimens inactivated in TriReagent LS to evaluate viral population distributions based on the sequence of EBOV GP. The data will be compiled and submitted as a contributing scientist report.

Results

Survival

There was a dose-dependent impact on survival (through Day 29) with 100% survival among the nine NHPs treated with 150 mg/kg REGN-EB3 compared to eight of nine treated with 100 mg/kg REGN-EB3 (88.9%) and seven of nine treated with 50 mg/kg REGN-EB3 (77.8%). Only four of nine NHPs survived in the 10 mg/kg group (44.4%) and there were no survivors in the placebo group (n=4) (Figure 42). However, one NHP was FDIC on Day 30 in the 150 mg/kg group (scheduled termination days 35 to 36) and one NHP in the 100 mg/kg cohort was found dead in cage on Day 39 (scheduled termination days 119 to 121).

Figure 42. Kaplan-Meier plot of Survival by REGN-EB3 Dose



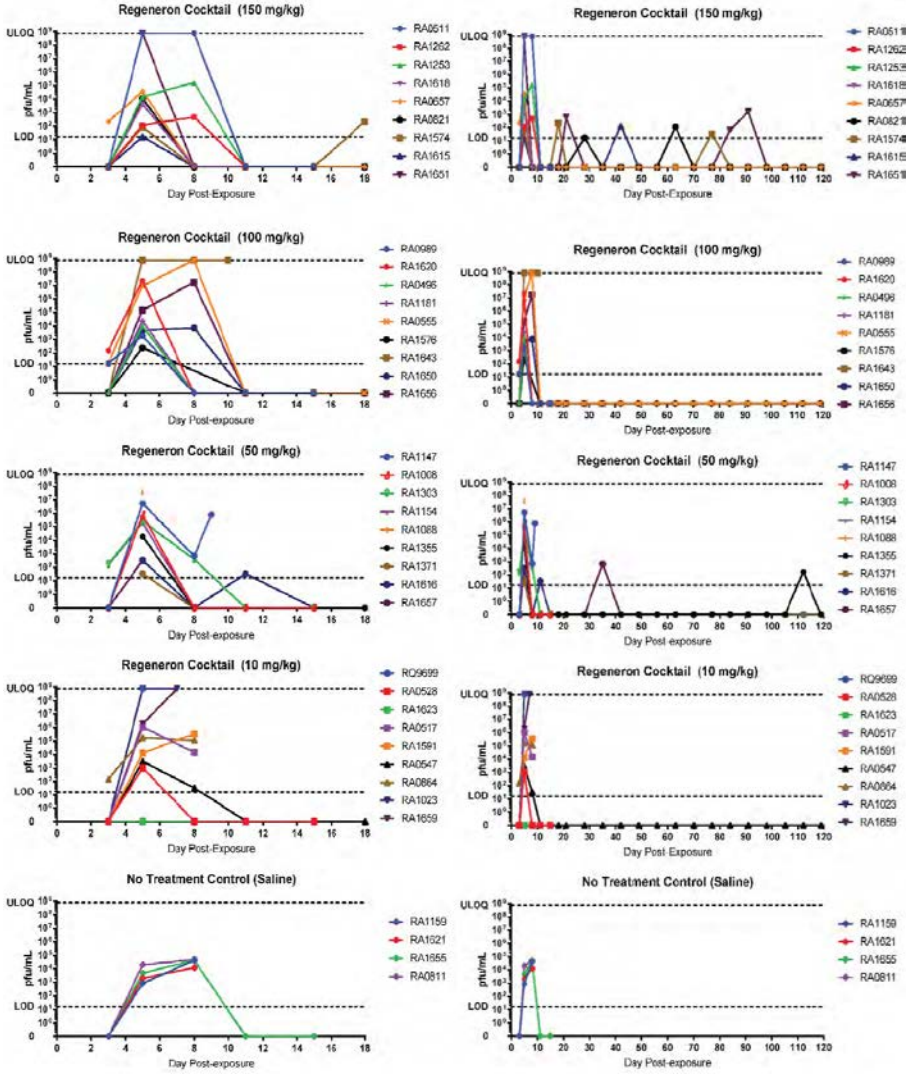
Source: Recreated from Figure 1, page 36, Study report AP-14-017 (IX and X)
Abbreviation: PBO, placebo

Animal RA1618 (150 mg/kg REGN-EB3) succumbed to EBOV infection on Day 30. For this animal, there was no evidence of typical active EBOV infection in target organs (spleen, liver, and/or lymph nodes). However, there was significant gastric ulceration and cerebral inflammation associated with the meninges, choroid, and ventricles. The inflammation in the brain was positive for EBOV antigen by immunohistochemistry; the lesions were significant and contributed to its death. Animal RA1656 (100 mg/kg REGN-EB3) succumbed to EBOV infection on Day 39, did not have typical lesions of EBOV in the target organs, but did have severe meningoencephalitis with vasculitis that was positive for EBOV antigen and RNA by immunohistochemistry and in situ hybridization, respectively.

Assessment of Serum EBOV and EBOV RNA

Serum EBOV detected by plaque assay. All animals (both studies) underwent viremia assessment on days 3, 5, 8, 11, 15, and prior to unscheduled euthanasia, whenever possible. NHPs in study AP-14-017 X also underwent viremia assessment on days 18, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, and 119 following EBOV exposure. In the 150 mg/kg group, eight of nine NHPs tested negative for serum EBOV on Day 3; animal RA0657 had a low concentration (2.17×10^2 PFU/mL) of EBOV. On Day 5 postexposure, all animals in the group showed evidence of circulating virus, with concentrations ranging from approximately 10^2 to 10^6 PFU/mL to too numerous to count. On Day 8, six of nine animals were negative for circulating EBOV with six animals showing sharp declines from Day 5 (day of treatment initiation) to Day 8 and three animals remaining positive (RA0511, RA1262, and RA1253). On days 11 and 15, all animals in this group were negative for circulating EBOV (Figure 43).

Figure 43. Serum EBOV Titers by Plaque Assay for the Dose Groups

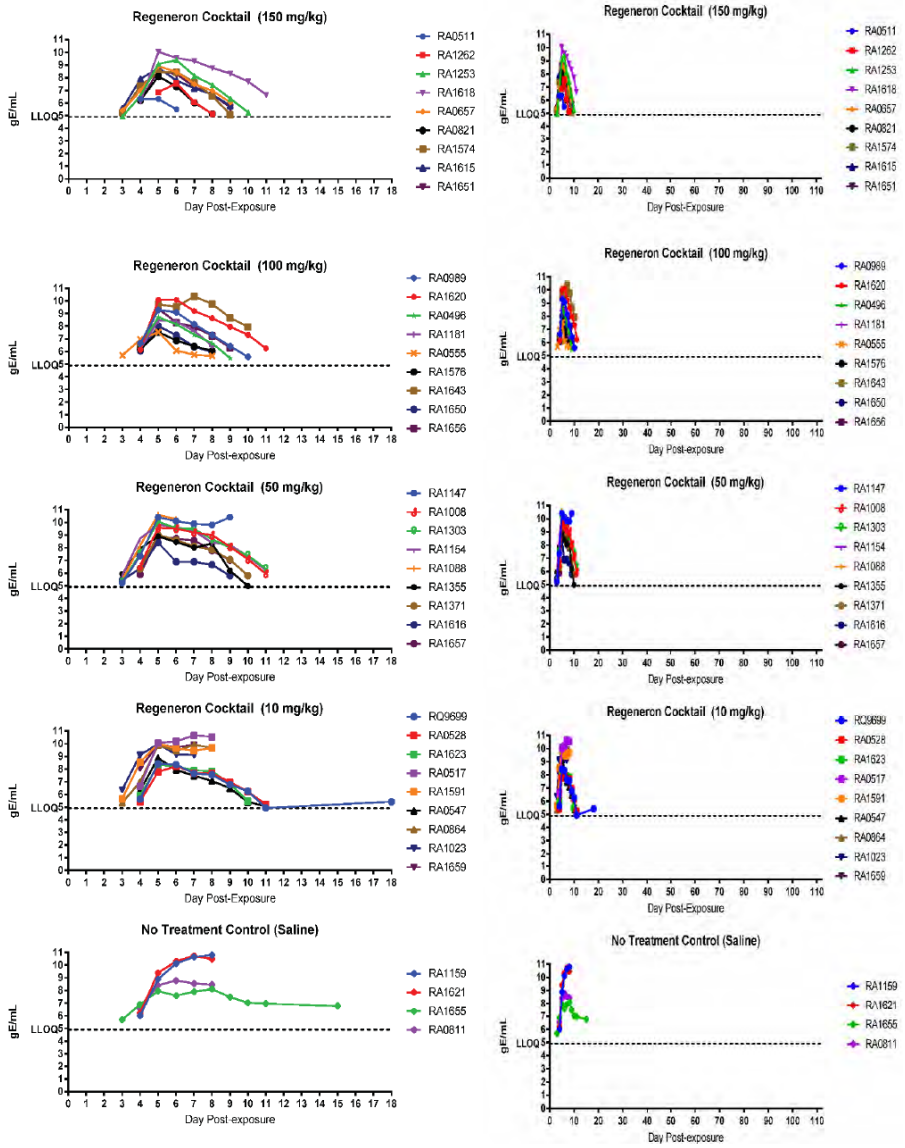


Source: Figure 2, page 41, Study report AP-14-017 (IX and X).
Graphs on left represent days 0 to 18 and graphs on right represent days 0 to 119. The ULOQ and LOD are represented as a dotted line across the graph.
Abbreviations: EBOV, *Zaire ebolavirus;* LOD, limit of detection; ULOQ, upper limit of quantification

Six of nine animals in the 100 mg/kg group tested negative for circulating EBOV on Day 3 while animals RA0555, RA0989, and RA1620 had detectable concentrations of approximately 10^1 to 10^4 PFU/mL. On Day 5 postexposure, all animals in the group had detectable serum EBOV, with concentrations of approximately 10^2 to 10^7 PFU/mL to too numerous to count. On Day 8, five of nine animals were negative for circulating virus and four remained positive. By days 11 and 15, all surviving animals (n=8) in this group were negative for circulating EBOV (Figure 43).

qRT-PCR for circulating EBOV genomes. Serum samples were collected from all animals (both studies) to assess circulating EBOV genomes on days 0 to 11, 15, 18, 21, 28, and 35. In addition, for AP-14-017 X, samples were also assessed on days 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, and 119 following EBOV exposure. The LLOQ was 4.9031 $\log_{10}$ GE/mL and values below the LLOQ were not plotted. In the 150 mg/kg group, seven of the nine NHPs had detectable circulating EBOV genome equivalents on Day 3 postexposure; four animals ranged from 4.94 to 5.56 $\log_{10}$ GE/mL, while three animals were only qualitatively positive (values were below the LLOQ) (Figure 44).

Figure 44. EBOV GE/mL by qRT-PCR for the Different Dose Groups



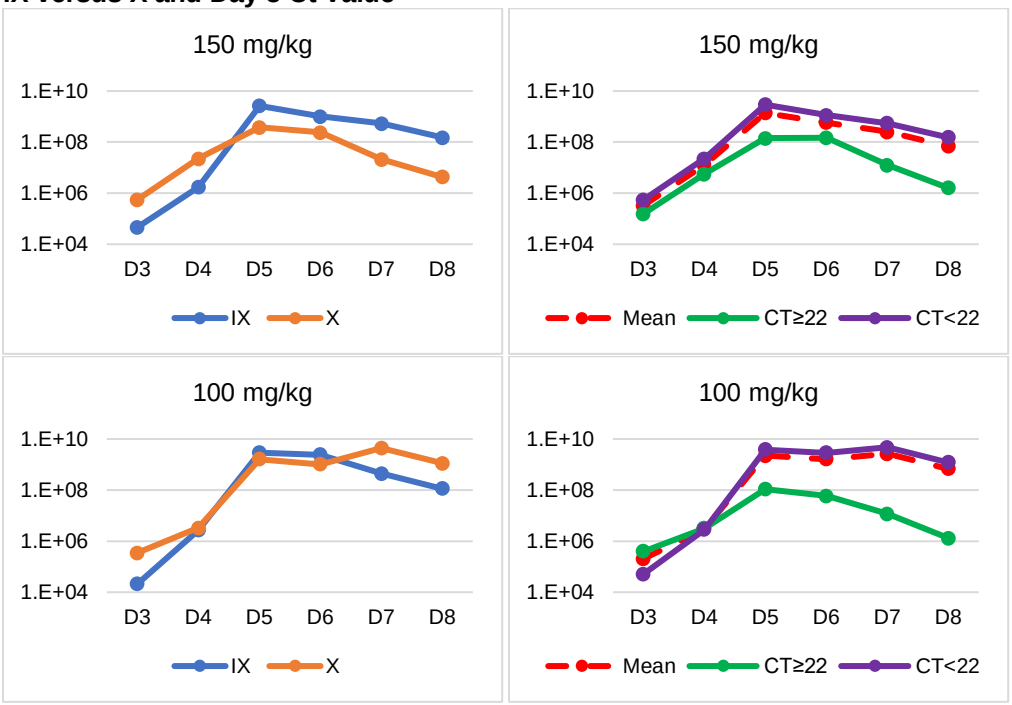
Source: Figure 2, page 41, Study report AP-14-017 (IX and X).
Graphs at left represent days 0 to 18 and graphs at right represent days 0 to 119. The ULOQ and LLOQ are represented as dotted lines.
Abbreviation: EBOV, *Zaire ebolavirus;* LLOQ, lower limit of quantitation; qRT-PCR, quantitative reverse transcription-polymerase chain reaction

By Day 5, prior to antibody treatment, all animals in this group were positive with maximal or near maximal levels of circulating viral genome equivalents of 6.31 to 10.02 $\log_{10}$ GE/mL. By Day 10, five animals had no detectable circulating viral genome equivalents, two animals were qualitatively positive, and two animals (RA1253 and RA1618) were positive with EBOV genome equivalent levels of 5.25 and 7.69 $\log_{10}$ GE/mL, respectively. These two animals had undetectable EBOV RNA levels on days 11 and 15 postexposure, respectively.

A comparison of the 150 mg/kg and 100 mg/kg dose groups based on study AP-14-017 IX versus X and based on the real-time RT-PCR Ct value of the samples processed on Day 5 (the day of drug administration) was performed to assess differences in GE/mL based on these

parameters (Figure 45). For the 150 mg/kg group, NHPs in study AP-14-017 IX had higher baseline EBOV GE/mL values prior to dosing with REGN-EB3 (Figure 45).

Figure 45. Comparison of the 150 mg/kg and 100 mg/kg Dose Groups Based on Study AP-14-017 IX versus X and Day 5 Ct Value

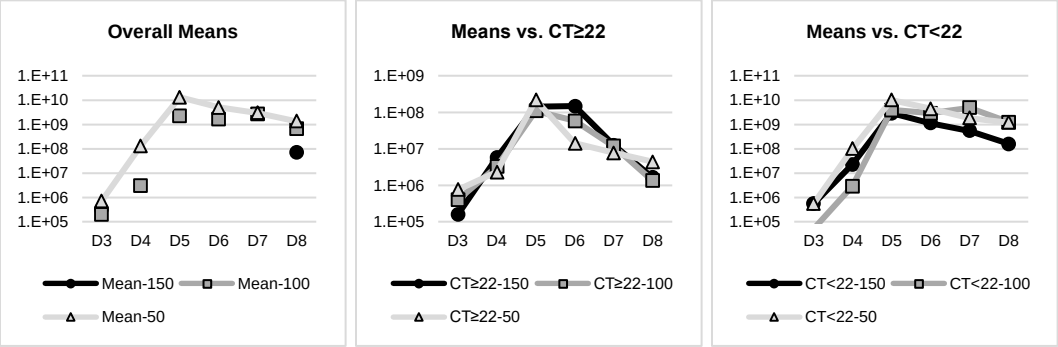


Source: DAV Analysis
Abbreviations: Ct, cycle-threshold; D, day

A Ct value of 22 was imputed by analyzing the NHP RT-PCR data to determine that a Ct value of 22 was equal to approximately 3.56×10^8 GE/mL. The NHPs from both studies (IX and X) in the 100 and 150 mg/kg dose groups were subdivided into groups based on Day 5 (baseline prior to drug administration) Ct <22 or Ct ≥22, and the mean GE/mL reductions were plotted for the overall group and the two Ct groups (Figure 45, right panels). In both cases, the Ct <22 group was largely responsible for the increased overall mean GE/mL values, and EBOV RNA reductions were greater in the Ct ≥22 group for both doses, with Day 5 to Day 8 EBOV RNA reductions of approximately 1 $\log_{10}$ in the Ct <22 groups and approximately 2 $\log_{10}$ GE/mL in the Ct ≥22 group (Figure 45, right panels).

Next, the overall mean EBOV RNA level reductions and the reductions in the Ct <22 and Ct ≥22 groups were compared among the 150 mg/kg, 100 mg/kg, and 50 mg/kg dose groups (Figure 46).

Figure 46. Comparison of Overall Mean GE/mL for the 150 mg/kg, 100 mg/kg, and 50 mg/kg Dose Groups and Means Based on Day 5 Ct Value



Source: DAV Analysis
Y-axis is EBOV RNA in GE/mL, X-axis is day postchallenge (challenge was on Day 0)
Abbreviation: Ct, cycle-threshold; D, day

Overall, the 150 mg/kg dose resulted in the greatest reductions in EBOV RNA (Figure 46, left panel); however, when comparing reductions based on Day 5 Ct values, the 100 mg/kg and 150 mg/kg groups performed equally well, with Day 5 to Day 8 EBOV RNA reductions of approximately 2 $\log_{10}$ GE/mL. The 150 mg/kg dose performed better than the other doses against the Day 5 Ct <22 group; however, the reduction of ~1.3 $\log_{10}$ GE/mL was less than that observed in the Ct ≥22 group, indicating that a dose higher than 150 mg/kg would be more effective in NHPs with EBOV RNA Ct <22 at the initiation of treatment on Day 5. These data also show that the 100 mg/kg dose does not perform as well as the 150 mg/kg dose in NHPs.

Conclusions

REGN-EB3 administered by IV at 150, 100, or 50 mg/kg 5 days after challenge demonstrated >75% survival against EBOV in a rhesus macaque lethal-challenge model. The Applicant stated that because the survival rates in the three highest REGN-EB3 dose groups (150, 100, and 50 mg/kg) were similar, the minimum effective concentration for treatment is 50 mg/kg REGN-EB3 when given IV on Day 5 postchallenge. However, when the data were evaluated based on Day 5 predose EBOV RNA (Ct values) reductions, the Ct ≥22 group treated with 100 mg/kg and 150 mg/kg performed equally well, with similar Day 5 to Day 8 EBOV RNA reductions. The 150 mg/kg dose performed better than the other doses against the Day 5 Ct <22 group; however, the reduction of ~1.3 $\log_{10}$ GE/mL was less than that observed in the Ct ≥22 group, indicating that a dose >150 mg/kg would be more effective in NHPs with serum EBOV RNA Ct <22 at the initiation of treatment on Day 5. These data show that the 100 mg/kg dose does not perform as well as the 150 mg/kg dose in NHPs, providing evidence that 50 and 100 mg/kg are not the optimal doses in NHPs.

Study Title: Efficacy studies of a monoclonal antibody cocktail against lethal challenges of Zaire Ebola virus strain Kikwit

Study Number: AP-14-017-IV-R3479-MX-15044

Objectives: The objective of this study was to evaluate the antiviral activity of REGN-EB3 compared to ZMapp in rhesus macaques to determine the level of protection against IM EBOV challenge.

Institute that conducted the study: USAMRIID

Animals: Eighteen research-naïve adult rhesus macaques of Asian origin, ten males and eight females, were included in the study. The NHPs had a starting age range of 3 to 5 years and an initial body weight range of 3.9 to 6.9 kg on Day 0 (day of infection).

Challenge strain: The EBOV Kikwit strain (lot #R4415; 7U 95%; AY354458) was derived from a 65-year-old female during an outbreak that occurred in 1995 in the DRC (formerly Zaire). The patient exhibited disease, was hospitalized, and died.

Euthanasia Criteria

Primary Euthanasia Criteria

Primary euthanasia criteria assessments were performed daily, and two to five assessments were performed during the critical disease phase (Days 7 to 11). Primary criteria scores were determined as follows:

0=alert, responsive, normal activity, free of disease signs or exhibits only resolved/resolving disease signs.

1=slightly diminished general activity, subdued but responds normally to external stimuli.

2=withdrawn, may have head down, fetal posture, hunched, reduced response to external stimuli.

3=prostrate but able to rise if stimulated or moderate to dramatically reduced response to external stimuli.

4=persistently prostrate, severely or completely unresponsive, may have signs of respiratory distress.

These criteria enabled the study staff to euthanize 100% of the study population directly before the NHPs succumbed to Ebola virus disease.

Secondary Euthanasia Criteria

Not provided in the study report. Appendix 15, which is referred to in the study report, was not included in the submission and Appendix K did not describe the secondary criteria.

Experimental Design

Study personnel were blinded to avoid bias pertaining to the identity of the study groups (control vs. treatment). On Day 0, rhesus macaques were IM injected in the right caudal thigh with 900 PFU EBOV R4415 (7U). On days 5, 8, and 11, all animals were treated by IV via a peripheral vein injection in a single slow bolus push over at least 30 seconds with a 15 mL total volume that contained saline (n=4), 50 mg/kg of the original ZMapp™ cocktail produced in tobacco leaves (n=4), 50 mg/kg of Regeneron's ZMapp™ cocktail produced in CHO cells, or 50 mg/kg of REGN-EB3 (labeled REGN1 in some figures). The antibody cocktails comprised the three mAb components at a 1:1:1 ratio. REGN-EB3 comprised a 1:1:1 ratio of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479).

The primary endpoint of this study was survival to Day 28. Additional parameters evaluated included: physical observations, body weight; body temperature; hematology and clinical chemistry parameters; viral RNA level by qRT-PCR; viral genome sequencing; and anatomic pathology, including macroscopic and microscopic observations, and immunohistochemistry procedures.

Virologic Assessments

Blood was collected on days -6, 0, 3, 5, 8, 11, 14, 21, and at the end of study (Day 28+). Blood was also collected from moribund animals to determine euthanasia criteria and/or prior to euthanasia. Collected blood samples were used to assess hematology and clinical chemistry parameters, viremia by plaque assay, and circulating viral genomes by qRT-PCR. The endpoint was euthanasia when moribund or at the end of study (Days 29 to 34). Necropsies were conducted on all animals and protocol-specified tissues were collected for histopathology examinations and virologic analyses.

1. Mortality: The primary endpoint survival ≥28 days postchallenge. Mortality was defined as the day on which an animal was FDIC or the day an animal was euthanized according to the criteria used by the study institution. All animals in this study were euthanized.
2. Plaque assay for serum EBOV RNA: All animals in the study underwent assessment of viremia on days 3, 5, 8, 11 and/or at the end of study.
3. qRT-PCR for circulating EBOV genomes: Serum samples were collected from all animals to assess circulating EBOV genomes on days 0, 3, 5, 8, 11, 14, 21, and at the end of study (Day 28+) following EBOV exposure. The LLOQ for the RT-PCR assay was 4.9031 log10 GE/mL.
4. Viral genome sequencing: The protocol indicated that viral genome sequencing would be conducted, but no data were included in the study report.

Virology Assays

1. **qRT-PCR**: Inactivated samples were extracted and eluted with AVE Buffer using a QIAamp Viral RNA Mini Kit. The samples were subjected to qRT-PCR on an ABI 7500 Fast Dx. The RT-PCR reaction used the SuperScript II One-Step RT-PCR System (Invitrogen) with additional MgSO4 to a final concentration of 3.0mM. The sequence of the primer and probe for the EBOV GP gene were:
    a. Forward primer (1μM): 5′-TTT TCA ATC CTC AAC CGT AAG GC-3′
    b. Reverse primer (1μM): 5′-CAG TCC GGT CCC AGA ATG TG-3′
    c. Probe (0.1μM): 6FAM-CAT GTG CCG CCC CAT CGC TGC–TAMRA
    d. The GE values were determined using a standard curve of synthetic RNA of known concentration. The LLOQ was 4.9031 log10 GE/mL.
    e. ***Note:*** *The investigator noted that on May 14, 2015, the temperature of the freezer went out of the protocol-specified range (protocol deviation #14). The temperature was recorded at -18.8°C for 15 minutes. There was no impact on the study because the samples were maintained frozen and did not thaw.*
2. **Plaque Assay:** The LOD and LLOQ for the plaque assay were not provided but are presumably the same as previous studies conducted at USAMRIID (LOD 16.5 PFU/mL, LLOQ 500 PFU/mL, and upper limit of quantitation 7.5×108 PFU/mL).
3. **Viral Genome Sequencing:** Not performed.

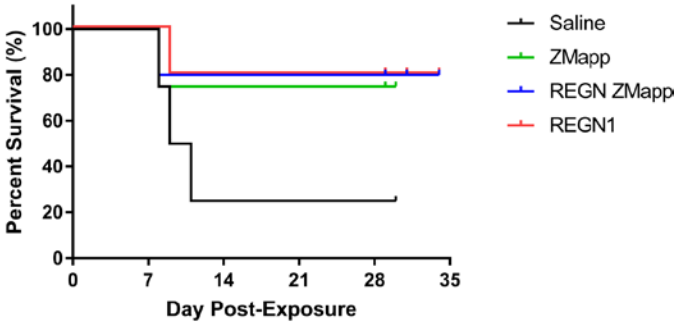
Results

Survival

Three of the four control NHPs succumbed to EBOV infection, for a 25% survival rate. One animal from each treated group succumbed to EBOV infection (Figure 47). Survival for the original ZMapp™ treatment group was 75% (3/4); in the Regeneron CHO-derived ZMapp™ group 80% (4/5) of NHPs survived, and in the REGN-EB3 group 80% (4/5) of NHPs survived to

Day 28. There was a significant difference between the saline control group and each of the treatment groups (Figure 47).

Figure 47. Survival by Treatment Group

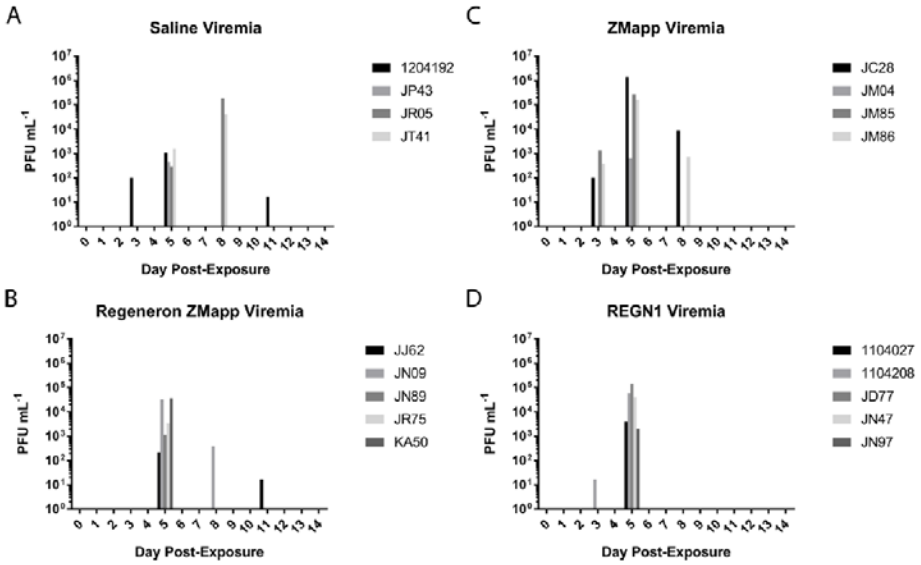


Source: Figure 1, page 39, Study report AP-14-017-R3479-MX-15044

Assessment of Serum EBOV and EBOV RNA

Plaque Assay for Viremia. All animals in the study underwent assessment of viremia by plaque assay on days 3, 5, 8, 11 and/or at the end of study (Day 28+) following EBOV exposure (Figure 48). For REGN-EB3, one NHP had viremia on Day 3; however, before treatment on Day 5, all five NHPs in this group had a circulating virus level of 2,030 to 140,000 PFU/mL. After treatment on Day 8, viremia was not detected in any of the NHPs in this group (Figure 48). For the original ZMapp™ group, three of four NHPs had circulating virus on Day 3 (100 to 1,325 PFU/mL). All four animals had increased viremia on Day 5 compared to Day 3 (667 to 1,433,333 PFU/mL). After treatment on Day 5 all four animals experienced a decline in viremia with two of the animals having no detectable viremia. The animals that were positive on Day 8 had titers of 717 to 8,667 PFU/mL. None of the three surviving animals had positive titers for the remainder of the study. For the Regeneron CHO-derived ZMapp™ group, all five NHPs tested negative for circulating virus by plaque assay until Day 5 postchallenge. On Day 5, all animals had circulating virus (approximately 217 to 36,833 PFU/mL). On Day 8, four of the five animals had no detectable circulating virus; only NHP JN09, which succumbed on this day, had a circulating virus level of 383 PFU/mL. The Day 8 titers, when detectable, were greatest in the saline control group (Figure 48).

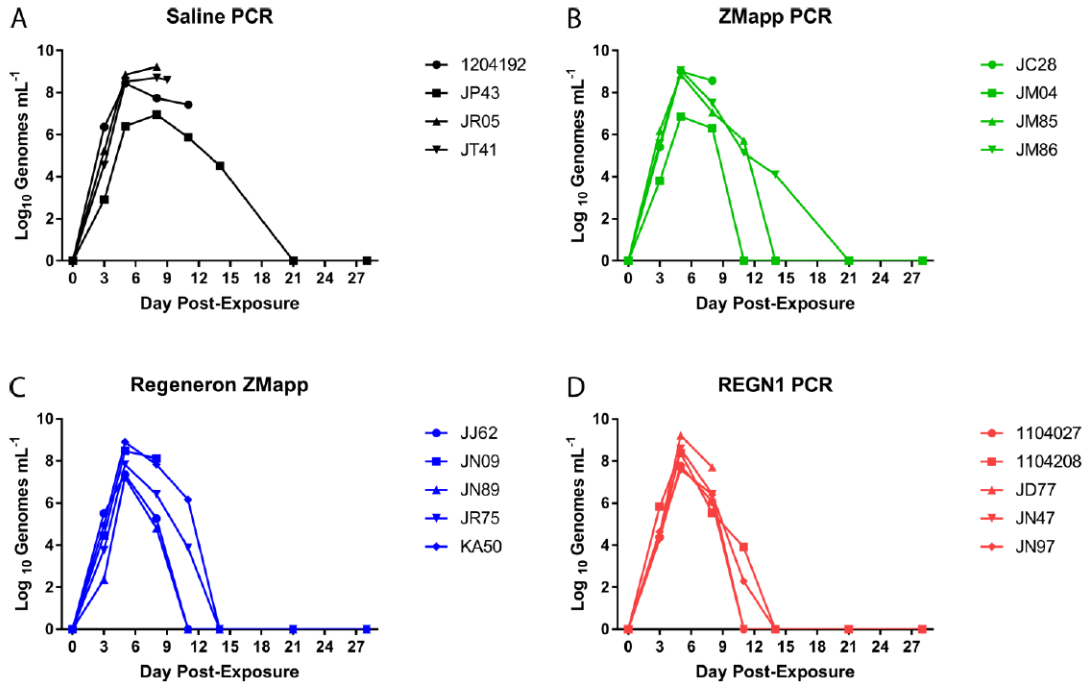
Figure 48. Viremia Assessed by Plaque Assay for Each Treatment Group



Source: Figure 2, page 41, Study report AP-14-017-R3479-MX-15044
A) Saline treatment group; B) Regeneron CHO-derived ZMapp™; C) Original ZMapp™; D) Regeneron's REGN1 cocktail
Abbreviation: PFU, plaque-forming unit

qRT-PCR for circulating EBOV genomes. All animals had serum samples collected to assess circulating EBOV genomes on days 0, 3, 5, 8, 11, 14, 21, and at the end of study (Day 28+). For the REGN-EB3 group, all NHPs were positive by qRT-PCR for EBOV RNA on Day 5, when antibody treatment began. Two animals were qualitatively positive on Day 3, while only one animal was quantitatively positive with a GE of 5.84 $\log_{10}$ GE/mL. The range of circulating viral GEs per mL on Day 5 was 7.60 to 9.22 $\log_{10}$ GE/mL. On Day 8, the range of circulating GEs was 5.54 to 7.70 $\log_{10}$ GE/mL. On Day 11; two of the four surviving animals had no detectable circulating viral GEs and two animals were below the LLOQ. By Day 14, there were no detectable circulating viral GEs in the surviving NHPs. Cage 5/JD77, which succumbed to disease on Day 9, had a viral GE of 7.43 $\log_{10}$ GE/mL (Figure 49).

Figure 49. EBOV GE/mL Based on qRT-PCR for the Different Treatment Groups



Source: Figure 3, page 43, Study report AP-14-017-R3479-MX-15044
A) Saline treatment group; B) Original ZMapp™; C) Regeneron CHO-derived ZMapp™; D) Regeneron's REGN1 cocktail, REGN-EB3
Abbreviations: EBOV, *Zaire ebolavirus;* PCR, polymerase chain reaction; qRT-PCR, quantitative reverse transcription-polymerase chain reaction

For the original ZMapp™ group, all NHPs were positive via qRT-PCR for Ebola virus on Day 5 with a range of circulating viral GE/mL on Day 5 of 6.86 to 9.06 $\log_{10}$. On Day 8, the animals in this group showed only slight reductions in circulating viral GEs of 6.30 to 8.31 $\log_{10}$ GE/mL. On Day 11, two of the three surviving animals had circulating viral GEs of 5.13 to 5.72 $\log_{10}$ GE/mL. By Day 14, there remained no detectable circulating viral GEs in the surviving NHPs. Cage 4/JC28, which succumbed to disease on Day 8, had a viral GE of 8.57 $\log_{10}$ GE/mL.

For the Regeneron CHO-derived ZMapp™ group, all NHPs were positive by qRT-PCR for EBOV RNA on Day 5 with a range of 7.22 to 8.90 $\log_{10}$ GE/mL. On Day 8, the range of circulating GEs was 5.26 to 8.12 $\log_{10}$ GE/mL. On Day 11, two of the four surviving animals had no detective circulating viral GEs, one animal was below the LLOQ, and the remaining animal, KA50, had a circulating viral GE of 6.16 $\log_{10}$ GE/mL. By Day 14, there remained no detectable circulating viral GEs in the surviving NHPs. Cage 10/JN09, which succumbed to disease on Day 8, had a viral GE of 8.12 $\log_{10}$ GE/mL.

For the control group, all NHPs tested positive by Day 5, with a range of circulating GE/mL of 6.40 to 8.85 $\log_{10}$. The maximal levels of GE/mL occurred from days 5 to 8 and ranged from 6.95 to 9.23 $\log_{10}$ GE/mL.

Conclusions

The results from this study indicate that treatment of rhesus macaques with the original ZMapp™ antibody cocktail, Regeneron CHO cell-derived ZMapp™ cocktail, and the REGN-EB3 cocktail had an effect on EBOV infection and allowed >75% of animals in each group to survive EBOV challenge. There was no statistically significant difference in survival between

the treatment groups. In each of the cocktail groups, one animal succumbed to EBOV infection. Each of the animals that succumbed had very high levels of circulating viral genomes and virus.

Study Title: Efficacy Studies of a Monoclonal Antibody Cocktail Against Lethal Challenges of Nonhuman Primates with EBOV

Study Number: (b) (4) 2015-002-PD-01v1-Amend4

Objectives: To evaluate the antiviral activity of different doses and regimens of REGN-EB3 in a time-to-treat study in a NHP IM EBOV lethal challenge model.

Institute that conducted the study: (b) (4)

Animals: Eighteen experimentally naïve, male rhesus macaques 3 to 6 years of age of weight 4.0 to 6.9 kg were used in this study. Only males were used due to the availability of animals at the time of procurement.

Challenge strain: Wild-type EBOV Kikwit (9510621) with only 1 SNP (GP substitution T544I; (Hoffmann et al. 2017; Ruedas et al. 2017; Ueda et al. 2017) difference from the passage 2 (P2) consensus sequence. The P3 virus was 95% 7U, compared to the P2, which was 98% 7U (uracil). Titer at harvest: 2.1×10^5 PFU/mL.

Euthanasia Criteria

The key endpoint in this study was survival/nonsurvival. Nonsurvival was defined by an animal having terminal illness or being moribund using pre-established criteria. Animal health was evaluated on a daily clinical observation score sheet. The total clinical score was determined by summing all of the clinical scores. Clinical scores were reported to the responsible veterinarian daily and documentation of the veterinarian review was maintained in the study file. If the clinical score was ≥8, the animal was reported to the study veterinarian for immediate evaluation. If the total clinical score was ≥15, the animal was considered terminally ill and was euthanized upon approval by the responsible veterinarian.

Additional euthanasia criteria: Animals were humanely euthanized as soon as any of the following were observed: 1) Prostrate but able to rise if stimulated, moderate to dramatically reduced response to external stimuli along with a ≥5°F change from baseline or 2) prostrate but able to rise if stimulated, moderate to dramatically reduced response to external stimuli along with any two of the following (during the most recent blood draw): ALT >200; ALP >1100; GGT >170; BUN >30; and ALB <3.0.

The veterinarian approved all euthanasias and documentation of this approval was maintained in the study file.

Experimental Design: The objective of this blinded study was to screen for the antiviral activity of REGN-EB3 mAb in rhesus macaques. Animals were challenged by injection in the right deltoid muscle of the arm with a target dose in the range of 1,000 PFU (actual 1,270 PFU) of EBOV. The study involved challenging 18 rhesus macaques, which were assigned to the experimental groups listed in Table 108.

Table 108. Experimental Groups

Group	Blinded Group Name	Group Description	Time of Dosing relative to Exposure (concentration)	Exposure EBOV WT 7U IM
1	RG11 (n=4)	REGN-EB3	Day 5, 8, 11 (50 mg/kg)	1000 PFU
2	RG12 (n=5)	REGN-EB3	Day 5 and 8 (50 mg/kg)	1000 PFU
3	RG13 (n=5)	REGN-EB3	Day 5 (150 mg/kg)	1000 PFU
4	RG14 (n=4)	Placebo (normal saline)	Day 5, 8, 11 (50 mg/kg)	1000 PFU
Total	18			

Source: Modified from Table 2, page 23, Study Report (b) (4) 2015-002-PD-01v1-Amend4
Abbreviations: EBOV, *Zaire ebolavirus;* IM, intramuscularly; n, number of subjects in subgroup; PFU, plaque-forming unit

Blood (4.0 mL) was collected on Day -8 relative to virus exposure (Day 0) for serum and plasma isolation. Next, the animals were transferred to the animal biosafety level 4 laboratory. On Day 5, 8, or 11 per the study schedule (Table 108), NHPs received an IV dose of REGN-EB3 or placebo control material according to Table 108. The key endpoint in this study was survival. Nonsurvival was defined as an animal having terminal illness (as defined by euthanasia criteria) or being moribund. The animal's health was evaluated on a daily clinical observation score sheet. Clinical observations, body weight, body temperature, hematology, clinical chemistry, coagulation, viral load (blood and tissues), and macroscopic and microscopic pathology were assessed.

Virologic Assessments

Whole blood prior to and after treatment on treatment days was obtained from living NHPs on days -8, 0, 3, 5, 8, 11, 14, 21 and 27/28 (relative to exposure). In addition to the scheduled collections, blood was collected from monkeys considered to be moribund prior to euthanasia.

1. **Mortality:** The primary endpoint was survival at ≥28 days postchallenge. Mortality was defined as an animal having terminal illness or being moribund using pre-established criteria. All animals in this study were euthanized. Of the 18 NHPs in the study that were considered nonsurvivors (n=6), 4 were euthanized (scores of 13 to 28), 1 was found dead in cage on Day 7 (placebo group), and 1 died during sedation on Day 7 (placebo group).
2. **Plaque assay for serum EBOV RNA analysis:** Viremia was analyzed using serum collected from Study Day 0 to the day of euthanasia. Viremia was determined by plaque assay (using a 500 µL aliquot of frozen serum) according to (b) (4) SOP V&I 4006. Results from the plaque assay were reported as PFU/mL of serum. Quantitative RT-PCRs of serum RNA were performed in duplicate.
3. **qRT-PCR for circulating EBOV genomes:** Viremia was analyzed using serum collected from Study Day 0 to the day of euthanasia. Viremia was determined by quantitative RT-PCR (100 µL) according to (b) (4) SOP V&I 2009 using serum RNA samples and was performed in duplicate.
4. **Tissue Viral Load.** Tissue samples from the spleen, liver, lung, axillary lymph node, and adrenal gland were taken at necropsy, and weighed and analyzed for the presence of filovirus by plaque assay according to (b) (4) SOP V&I 4006

and qRT-PCR. Genome quantification (qRT-PCR) from collected samples was conducted according to (b) (4) SOP V&I 2009. Quantitative results were reported as GE/µg tissue and infectious virus titers as PFU/g.

5. **Determination of sGP in serum.** At study days -8, 0, 3, 5, 8, 11, 14, 21, and 27/28 (relative to exposure), and at study termination, whole blood was collected from a vein into a plastic serum separator tube or equivalent and processed to obtain serum ((b) (4) SOP ICL-12 and (b) (4) SOP 901). The blood samples for sGP were collected 5 to 10 minutes prior to dosing with the Ab cocktail or placebo. Serum was assayed for sGP levels using an assay developed by (b) (4)
6. **Next-generation sequencing:** Tissue samples taken at necropsy were subjected to next-generation sequencing and analyzed for mutations in viral genomes that resulted in GP substitutions. Samples from immune-privileged sites (eyes, testes, brain) were sequenced using Illumina™ technology for mutations in the viral genome sequence. Additionally, serum samples from days 5, 8, and the day of euthanasia for fatal cases were tested.

Virology Assays

1. **qRT-PCR of serum and tissue samples (SOP V&I 2009):** Viremia was determined by qRT-PCR (100 µL) according to (b) (4) SOP V&I 2009, which was not provided in the study report. Quantitative RT-PCR of serum RNA was performed in duplicate and the results reported as GE/mL. The analysis of tissue homogenates for EBOV RNA by quantitative RT-PCR was performed on two replicates per sample. Quantitative results were reported as GE/µg of tissue and infectious virus titer was reported as PFU/g.
2. **FANG plaque assay of serum and tissue samples (SOP V&I 4006):** Viremia was determined by FANG plaque assay following SOP V&I 4006, which was not included in the study report. Results from the FANG plaque assay were reported as PFU/mL of serum. The LOD of the plaque assay was 25 PFU/mL.
3. **sGP assay:** The NHP sGP assay was designed to quantify sGP in NHP serum. The Applicant noted that an ELISA is commercially available that can measure sGP in serum but, because it is based on antibodies to sGP, serum from animals treated with the REGN cocktail would likely interfere with the assay. Therefore, the NHP sGP assay was designed to quantify sGP in samples that were denatured to disrupt sGP-antibody interactions. Samples were boiled in SDS-PAGE loading buffer in the presence of a reducing agent. The ELISA was not functional (weakly) under these conditions, so an immunoblot (Western blot) was performed instead. Samples were treated with PNGase F to remove carbohydrates from proteins to distinguish between sGP and an endogenous NHP serum protein of similar size. Immunoblots were then assayed using the LiCor system. The assay range was 0.3 µg/mL to 20 µg/mL. Those values were converted to sGP concentrations by multiplying the band intensity by the dilution factor; this yielded a range of 7 µg/mL to 381 µg/mL.
4. **NGS Genome Sequencing:** Ultradeep sequencing was performed using the Illumina MiSeq platform in an unbiased fashion and so the Applicant removed cellular chromosomal DNA, ribosomal RNA, and messenger RNA from the samples prior to sequencing. A protocol and detailed methodology were provided

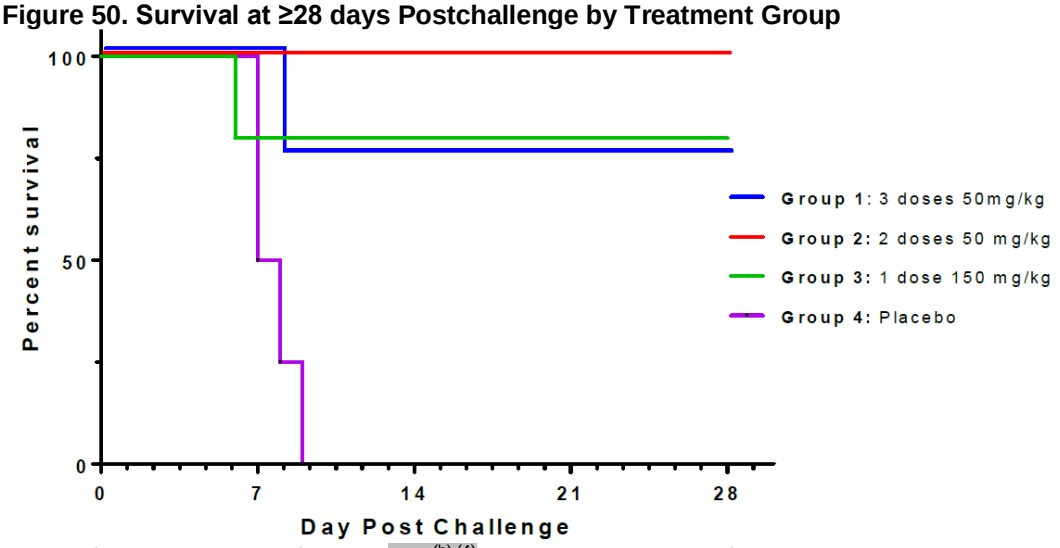
in the appendix of the study report, and overall this approach appeared to be robust. The Applicant provided a sample by sample detailed description of the sequencing runs, indicating which samples had problems and identifying those problems. Sequencing was performed for the entire EBOV genome and the sequence of the parental virus (EBOV Kikwit P3) was used as the reference sequence for mapping reads and calling variants. The fastq sequences were not provided for this assessment; therefore, an independent analysis was not performed.

Results

Survival

All five NHPs challenged with EBOV on Day 0 that received two doses of 50 mg/kg of REGN-EB3 on days 5 and 8 in RG12 (Group 2) survived to Day 28 (Figure 50). In RG13 (Group 3), four of five (80%) NHPs survived EBOV infection after a single dose of 150 mg/kg of REGN-EB3 administered on Day 5, and in RG11 (Group 1) three of four (75%) NHPs survived to Day 28 after receiving three doses of 50 mg/kg of REGN-EB3 on days 5, 8, and 11 (Figure 50). All four animals in the control group were euthanized by days 7 to 9.

Figure 50. Survival at ≥28 days Postchallenge by Treatment Group



Source: Figure 1, page 33, Study Report (b) (4) 2015-002-PD-01v1-Amend4

Assessment of Serum EBOV and EBOV RNA

EBOV was tested in serum and in tissues by FANG Plaque Assay and in serum by qRT-PCR.

Plaque Assay to Assess EBOV Viremia *(data provided but not shown)*

All animals in the study had viremia as assessed by plaque assay on days 0, 3, 5, 8, 11, 14, 21, 27/28 and/or at the end of study (Day 28+) following EBOV challenge. The Day 5 titers for NHPs of all groups that had a detectable titer (n=12) ranged from 3.55×10^2 to 1.69×10^6 PFU/mL. Six NHPs had an undetectable EBOV titer at Day 5. All NHPs with Day 5 titers $\geq3.60\times10^6$ PFU/mL succumbed to infection, including one animal each in groups RG11 and RG13. In addition, three NHPs in the placebo group (RG14) succumbed to infection but had an undetectable viral load on Day 5. No EBOV titers were detectable for any of the treated NHPs after Day 5, while two NHPs in the placebo group (RG14) had Day 8 titers of 1.40×10^6 and 1.01×10^6 PFU/mL. These NHPs were euthanized on days 8 and 9, respectively.

Plaque Assay to Assess EBOV in Tissues *(data provided but not shown)*

Samples from the spleen, liver, lung, axillary lymph node, and adrenal gland were taken at necropsy and assessed for EBOV by plaque assay. No EBOV was detected in the tissues of any NHP from any of the treatment groups. For the placebo group, EBOV was detected in all tissues, at titers of 7.69×10^5 to 3.25×10^6 PFU/g in the spleen, 1.14×10^6 to 1.92×10^6 PFU/g in the liver, 2.73×10^2 to 2.17×10^5 PFU/g in the lung, 3.00×10^5 to 1.49×10^7 PFU/g in the axillary lymph node, and 2.31×10^4 to 1.40×10^6 PFU/g in the adrenal gland.

qRT-PCR for Circulating EBOV Genomes

All animals had serum samples collected to assess circulating EBOV genomes on days 0, 3, 5, 8, 11, 14, 21, and at the end of study (Day 28+) (Table 109). Animals that received treatment with REGB-EB3 that succumbed to infection (n=2 NHPs) had Day 5 EBOV titers $>1\times10^8$ GE/mL (Table 109). None of the NHPs in RG12 (Group 2) had Day 5 EBOV titers $>9.22\times10^6$ GE/mL (range 2.81×10^5 to 9.22×10^6 GE/mL) and all NHPs in this group survived (Table 109). Day 5 EBOV titers in RG13 (Group 3) ranged from 2.22×10^5 to 3.50×10^8 GE/mL, and the NHP with the highest Day 5 titer was euthanized on Day 6. The Day 5 EBOV titers in RG11 (Group 1) ranged from 8.54×10^4 to 1.83×10^8 GE/mL, and the NHP with the highest titer was euthanized on Day 8.

Table 109. EBOV Viral Load by qRT-PCR of Serum Samples by Dose Group to Day 21

	Study Day						
Dose Cohort/NHP	**0**	**3**	**5**	**8**	**11**	**14**	**21**
RG13 1×150 mg/kg							
34928			4.16E +06	1.48E +05	1.19E +03		
34930	4.21E +03		2.22E +05	4.53E +05	1.02E +04	7.10E +02	
34933[a]		**1.51E +04**	**3.50E +08**				
34934		1.29E +03	1.13E +07	1.36E +06	6.42E +03		
34935		9.17E +02	4.05E +06	6.20E +05	6.87E +03		
RG12 2×50 mg/kg							
34924			7.13E +06	2.92E +06	4.66E +04		
34925	ND		1.92E +06	2.85E +05			
34937			2.81E +05	1.53E +04			
34938			9.22E +06	2.18E +05			
34939			7.60E +05	1.97E +06			
RG11 3×50 mg/kg							
34923			2.22E +06	1.59E +07	4.07E +05	1.95E +03	1.03E +03
34927			8.54E +04	N/A	1.10E +03		
34931[a]		**5.05E +03**	**1.83E +08**				
34936			1.42E +06	4.71E +05	1.85E +03	7.59E +02	7.07E +02
RG14 PLACEBO							
34922[a]			1.03E +05				
34926[a]			2.34E +04				
34929[a]		**1.57E +03**	**2.34E +08**				
34932[a]			4.35E +04	5.77E +07			

Source: Created from Table 10, page 53, Study Report (b) (4) 2015-002-PD-01v1-Amend4).
[a] Row indicates NHPs that died.
Bold type indicates RT-PCR results from NHPs with Day 5 titers >1×10^8 GE/mL.
Blank cells represent values that were undetermined (<10 GE/mL). N/A, sample not available. ND, not done
Abbreviations: qRT-PCR, quantitative reverse transcription-polymerase chain reaction

Assessment of sGP

Serum samples were collected on days -8, 0, 3, 5, 8, 11, 14, 21, and 27/28 (relative to exposure). The blood samples for sGP were collected 5 to 10 minutes prior to dosing with the mAb cocktail or placebo. Two NHPs that received REGN-EB3 treatment succumbed to infection during treatment (#34931 from the 3×50 mg/kg REGN-EB3 dose cohort and #34933 from the 1×150 mg/kg REGN-EB3 dose cohort). Analysis of the sGP for these two NHPs indicated that they had the highest sGP concentrations on Day 5 (prior to treatment) and the serum sGP concentration increased until the day prior to death, indicating that there could be a suboptimal odesivimab (REGN3471) concentration (Table 110).

Of note, based on studies in the Mechanism of Action section (Section III.18.1.2.1), atoltivimab (REGN3470) and odesivimab (REGN3471) have overlapping binding footprints within the GP region that defines sGP (residues 1 to 295); however, only odesivimab (REGN3471) had all footprint residues contained in the sGP portion of GP. The atoltivimab (REGN3470) footprint extended across the sGP/GP junction to include 11 additional residues (298 to 308) not contained in sGP. The binding studies in Section III.18.1.2.1 indicated that atoltivimab (REGN3470) does not bind sGP.

Table 110. sGP Concentrations of NHPs Treated With REGN-EB3 in (b) (4) 2015-002

	Study Day									
Dose Cohort/NHP	**0**	**3**	**5**	**6**	**8**	**9**	**11**	**14**	**21**	**28**
1 dose 150 mg/kg										
34928	<LOD	<LOD	9	nd	10	nd	7	7	<LOD	7
34930	<LOD	<LOD	8	nd	19	nd	7	7	7	7
34933[a]	<LOD	<LOD	**124**	**223**	**X**	nd	**nd**	**nd**	**nd**	**nd**
34934	<LOD	<LOD	8	nd	8	nd	8	<LOD	<LOD	<LOD
34935	<LOD	<LOD	<LOD	nd	31	nd	<LOD	<LOD	<LOD	<LOD
2 doses 50 mg/kg										
34924	<LOD	<LOD	7	nd	25	nd	8	<LOD	<LOD	<LOD
34925	<LOD	<LOD		nd	7	nd	7	7	7	7
34937	<LOD	<LOD	<LOD	nd	<LOD	nd	<LOD	<LOD	<LOD	<LOD
34938	<LOD	<LOD	<LOD	nd	28	nd	<LOD	<LOD	<LOD	<LOD
34939	<LOD	<LOD	<LOD	nd	58	nd	<LOD	<LOD	<LOD	<LOD
3 doses 50 mg/kg										
34923	<LOD	<LOD	12	nd	50	nd	20	12	11	11
34927	<LOD	<LOD	<LOD	nd	15	nd	8	7	7	7
34931[a]	<LOD	<LOD	**43**	nd	**71**	nd	**X**	**nd**	**nd**	**nd**
34936[a]	<LOD	<LOD	<LOD	nd	19	nd	<LOD	<LOD	<LOD	<LOD
PLACEBO										
34922[a]	<LOD	<LOD	**12**	nd	**X**	nd	**nd**	nd	nd	nd
34926[a]	<LOD	<LOD	**7**	nd	**39**	**X**	**nd**	nd	nd	nd
34929[a]	<LOD	<LOD	**204**	nd	**X**	nd	**nd**	nd	nd	nd
34932[a]	<LOD	<LOD	**7**	nd	**172**	**122**	**X**	nd	nd	nd

Source: DAV Analysis
[a] Row indicates NHPs that died.
Bold type indicates sGP results from NHPs that succumbed to infection. X indicates the NHP died prior to the timepoint.
Abbreviations: <LOD, below the limit of detection of the assay; Nd, not done; NHP, nonhuman primate; sGP, secreted glycoprotein

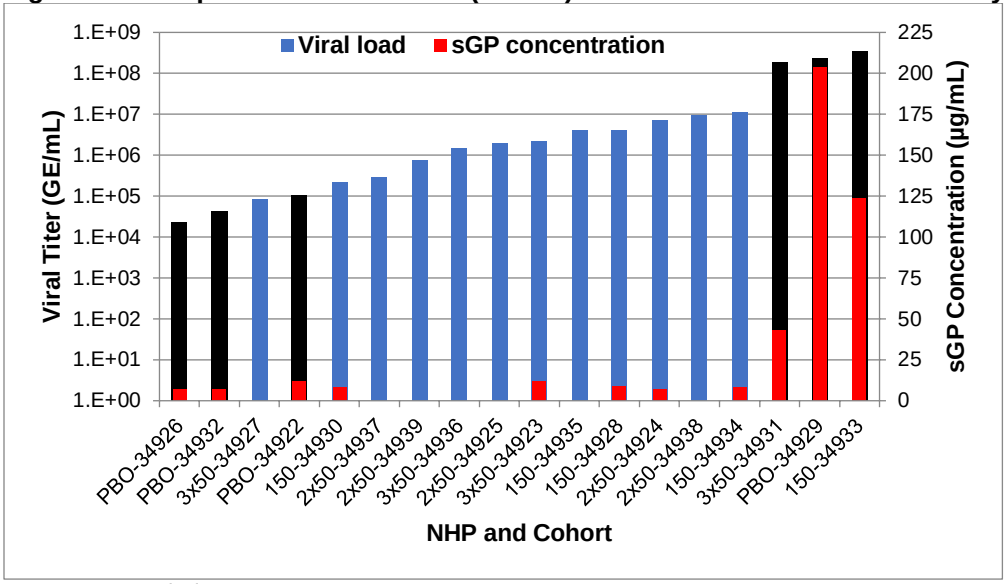
In addition, three of the other four animals in the 1×150 mg/kg group had little to no increase in sGP concentration; by contrast, the sGP concentrations increased from Day 5 to Day 8 among NHPs dosed with two or three doses of REGN-EB3 at 50 mg/kg (Table 110). The 150 mg/kg dose was more effective at reducing the sGP concentration overall. The investigator stated that

animals that succumbed to EBOV infection had significantly higher levels of sGP than those that survived (P<0.0004): Survivors=18±5 µg/mL; nonsurvivors=120±37 µg/mL.

In addition to the sGP concentrations, the viral loads at each study day were examined (Figure 51). The viral load at Day 5 in the two NHPs that received REGN-EB3 treatment and succumbed to infection were the highest among all treated animals; both were >1×10^8 GE/mL as measured by RT-qPCR (Figure 51).

Of note, a study of 183 patients infected with Ebola virus during the 2014 to 2015 outbreak who were treated at an Ebola virus treatment center in Guinea showed that a viral RNA load corresponding to a Ct of 19.5, or ~1×10^8 copies/mL (Miles Carroll seminar) or lower was significantly associated with a fatal outcome (Vernet et al. 2017) as seen in a Sudan outbreak (Towner et al. 2004). Importantly, based on the sGP data in humans (Sanchez et al. 1996; Cook and Lee 2013; de La Vega et al. 2015), it is possible that the concentration of odesivimab (REGN3471) in the cocktail is not sufficient to overcome the high concentration of sGP, and therefore, altering the cocktail to include a higher concentration of odesivimab (REGN3471) may be necessary for an optimal dose for patients with high titer (>1×10^8 GE/mL) and high sGP concentration (>40 µg/mL) EBOV infection.

Figure 51. Comparison of Viral Load (GE/mL) Versus sGP Concentration at Day 5



Source: DAV analysis
The graph is ordered from lowest to highest viral load at Day 5. The treatment cohort and NHP number are included on the X-axis with PBO=placebo group, 150=1×150 mg/kg dose group, 2×50=2×50 mg/kg dose group, and 3×50=3×50 mg/kg dose group. Black bars represent viral titers for NHPs that died, blue bars represent viral titers for NHPs that survived, red bars represent sGP concentration.
Abbreviations: NHP, nonhuman primate; sGP, secreted glycoprotein

The investigators also assessed sGP concentrations 10 minutes before and 10 minutes after dosing with the REGN-EB3 cocktail to assess the effect on sGP readout. In most cases there was no observable difference. However, in two cases there were major discrepancies that may be related to errors in the study report or to assay reproducibility (Table 111).

Table 111. sGP Concentrations in Samples Collected 10 Minutes Prior to Dosing and 10 Minutes After Dosing With REGN-EB3

	Before Dose on Study Day			After Dose on Study Day		
Dose Cohort/NHP	**5**	**8**	**11**	**5**	**8**	**11**
1 dose 150 mg/kg						
34928	9	10	7	<LOD	<LOD	<LOD
34930	8	19	7	<LOD	20	12
34933	124	X	nd	>381*	X	nd
34934	8	8	8	8	22	<LOD
34935	<LOD	31	<LOD	7	38	<LOD
2 doses 50 mg/kg						
34924	7	25	8	<LOD	33	7
34925	nd	7	7	nd	<LOD	<LOD
34937	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
34938	<LOD	28	<LOD	<LOD	28	<LOD
34939	<LOD	58	<LOD	<LOD	<LOD*	<LOD
3 doses 50 mg/kg						
34923	12	50	20	<LOD	38	23
34927	<LOD	15	8	<LOD	<LOD	<LOD
34931	43	71	X	159*	X*	nd*
34936	<LOD	19	<LOD	<LOD	27	<LOD
PLACEBO						
34922	12	X	nd	nd	nd	nd
34926	7	39	nd	nd	nd	nd
34929	204	X	nd	nd	nd	nd
34932	7	172	X	nd	nd	nd

Source: DAV Analysis
* Indicates major differences.
Abbreviations: <LOD, below the limit of detection of the assay; Nd, not done; sGP, secreted glycoprotein; X, results inconclusive

Assessment of NGS Data

The objective of this study was to investigate the EBOV genotypes in serum of infected NHPs at various times postinfection, and in selected tissues (such as immune privileged sites) harvested at necropsy. The goal was to characterize viruses that evolve in the presence of REGN-EB3 and that potentially escape therapeutic treatment.

Ultradeep sequencing was performed using the Illumina MiSeq platform in an unbiased fashion and so the Applicant removed cellular chromosomal DNA, ribosomal RNA, and messenger RNA from the samples prior to sequencing. A protocol and detailed methodology were provided in the appendix of the study report, and overall this approach appeared to be robust. The Applicant provided a sample by sample detailed description of the sequencing runs, indicating which samples had problems and identifying those problems. Sequencing of the entire EBOV genome was performed and the sequence of the parental virus (EBOV Kikwit P3) was used as the reference sequence for mapping reads and calling variants. The fastq sequences were not provided for this assessment; therefore, an independent analysis was not performed.

The Applicant provided results for the NGS data in frequency tables that reported the nucleotide variations and amino acid substitutions that occurred anywhere in the EBOV genomes sequenced from serum samples collected at Day 5 or end of study for all NHPs and in some cases from additional sites, such as the brain, prostate gland, testes, and vitreous humor. Variants were called based on differences between the sequence and the EBOV Kikwit P3 reference sequence. The data provided in the frequency tables were sufficient for review. For the analysis of the NGS data, data from only two NHPs were used for resistance analysis, from the two NHPs that died

while on treatment (NHP 34931 from the 3×50 mg/kg dose cohort and NHP 34933 from the 1x150 mg/kg dose cohort). This was because the virus from these two animals would be most likely to contain variants that could confer resistance to one or more mAbs in the cocktail. A summary of the NGS data for these two NHPs follows:

1. **NHP 34931 from the 3×50 mg dose cohort**—There were no genotypic differences in the EBOV GP from the last serum sample taken before death compared to the EBOV GP sequences from Day 5. The Applicant noted that there were insufficient data to generate variants from additional sites, such as the brain, prostate gland, testes, and vitreous humor.
2. **NHP 34933 from the 1×150 mg dose cohort**—There were several genotypic differences in the EBOV GP from the last serum sample taken before death compared to the EBOV GP sequences from Day 5 (Table 112) using a 2% reporting cutoff. However, all of these substitutions occurred at frequencies <5% and with coverages <200. The Applicant noted that there were insufficient data to call variants from the EBOV genomes sampled from additional sites, such as the brain, prostate gland, testes, and vitreous humor.

Table 112. Genotypic Differences in EBOV GP From the Serum of NHP 34933 at the Timepoint Prior to Death

NHP	Dose Group	Sample	DPI	AAPOS	SUB	AAFREQ	TCOV
34933	1×150 mg/kg	Serum	EOP	28	Q28L	0.034	58
34933	1×150 mg/kg	Serum	EOP	65	S65P	0.02	98
34933	1×150 mg/kg	Serum	EOP	222	A222G	0.022	87
34933	1×150 mg/kg	Serum	EOP	357	E357P	0.021	189
34933	1×150 mg/kg	Serum	EOP	427	P427A	0.021	95
34933	1×150 mg/kg	Serum	EOP	598	A598V	0.026	75
34933	1×150 mg/kg	Serum	EOP	605	D605N	0.03	66
34933	1×150 mg/kg	Serum	EOP	650	N650S	0.03	66

Source: DAV analysis
Abbreviations: AAPOS, amino acid position in GP; AAFREQ, frequency at which the substitution was found in all reads at the position; DPI, days postinfection; EBOV, *Zaire ebolavirus;* EOP, end of project (last evaluable timepoint prior to death); GP, glycoprotein; NHP, nonhuman primate; SUB, substitution detected; TCOV, total coverage

Overall, the results of the NGS analysis of EBOV GP in NHPs that died on treatment did not identify any substitutions that occurred at frequencies of ≥10% that could be associated with resistance. However, several substitutions with a frequency of <5% were reported, and these substitutions should be monitored for in future studies. None of the substitutions identified by NGS were close to the binding footprints of the REGN-EB3 cocktail (see section). Importantly, resistant virus need not account for a significant fraction because it could develop in one essential organ (leading to death) or animals may fail due to insufficient antibody to prevent virus replication.

Conclusions

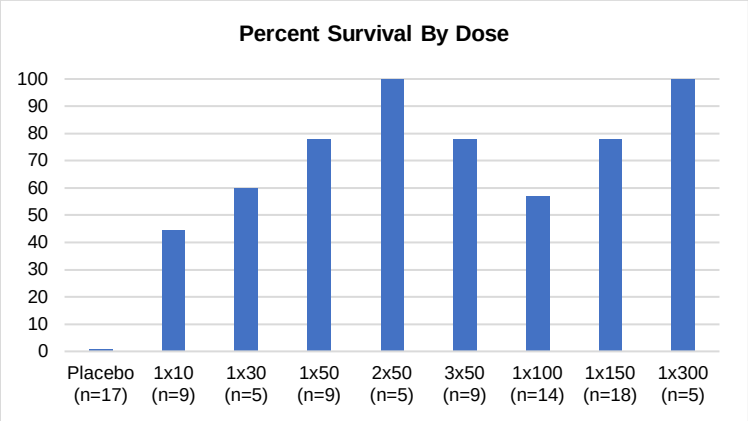
Treatment with REGN-EB3 starting 5 days after infection resulted in rescue of 12 of 14 NHPs challenged with a lethal dose of EBOV. Of the two treated NHPs that died, both had the highest Day 5 viral load, >8 $\log_{10}$ GE/mL, and both had the highest quantifiable sGP levels, >12 µg/mL. High Day 5 viral load and sGP concentrations were associated with mortality in this study. The results of the NGS analysis of EBOV GP in NHPs that died on treatment did not identify any substitutions that occurred at frequencies of ≥10% that could be associated with resistance.

However, several substitutions with a frequency of <5% were reported, none of which mapped to sites in the footprints of the three REGN-EB3 mAbs.

Overall Conclusions Based on NHP Challenge Studies

NHPs challenged with a lethal dose of EBOV and treated with REGN-EB3 at all doses assessed survived at a higher rate than those that received placebo (Figure 52). A total of 74 NHPs was challenged with EBOV and treated with REGN-EB3, with a dose-dependent response being observed from 10 to 50 mg/kg REGN-EB3.

Figure 52. Mean Percentage Survival Across the REGN-EB3 Development Program

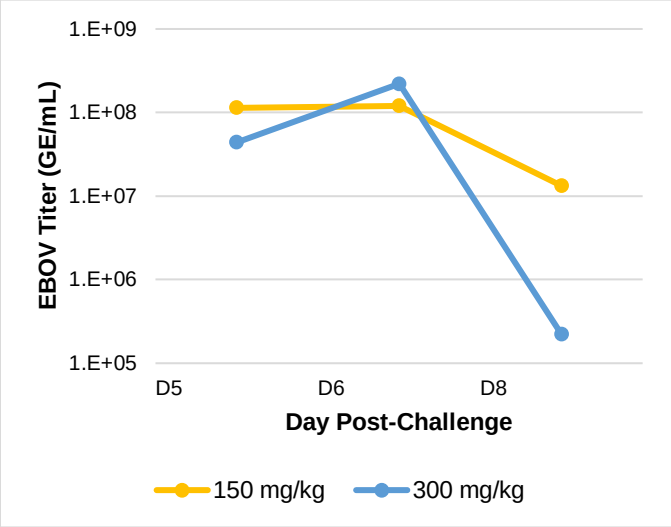


Source: DAV analysis
X-axis, dose; Y-Axis, percentage survival
Abbreviations: n, number of subjects in subgroup

At doses >50 mg/kg, variability in the NHP model and across study sites made it difficult to discern any real difference. For example, in study (b) (4) 2018-008 R3479-PM-18140, five NHPs were challenged with EBOV on Day 0 and treated with 1×100 mg/kg on Day 5 and only one NHP survived (20%). However, in studies AP-14-017 IX and X, four of four (100%) and four of five NHPs (80%) survived under the same experimental parameters; however, the studies were conducted at different research institutes.

In study (b) (4) 2018-008 R3479-PM-18140, a comparison of EBOV RNA decline from Day 5 (before initiation of treatment) to Day 8 showed a mean 1.2 $\log_{10}$ GE/mL decrease for the 150 mg/kg REGN-EB3 cohort (n=4 macaques) and a 2.1 $\log_{10}$ GE/mL decline for the 300 mg/kg REGN-EB3 cohort (n=5 macaques) (Figure 53).

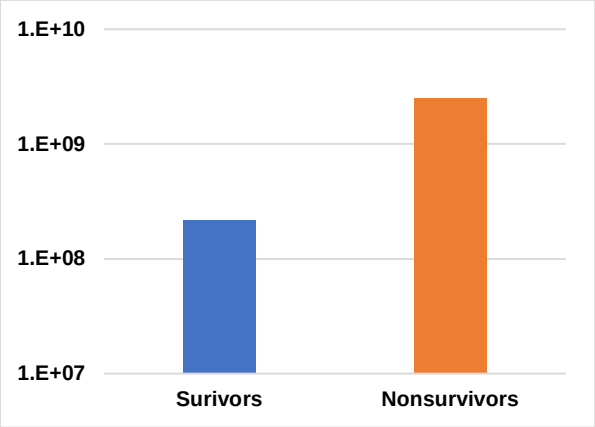
Figure 53. EBOV RNA Decline by Dose Comparing Single Doses of 150 mg/kg vs. 300 mg/kg



Source: DAV analysis
Abbreviation: D, day; EBOV, *Zaire ebolavirus;* RNA, ribonucleic acid

A total of 18 NHPs was treated with a single dose of 150 mg/kg REGN-EB3 administered 5 days after challenge and 14 of the 18 (77.8%) survived as a result of treatment. For the 14 NHPs that survived, the mean Day 5 titer was 2.16×10^8 GE/mL compared to a mean Day 5 titer of 2.54×10^9 GE/mL for nonsurvivors (Figure 54).

Figure 54. Mean EBOV Titer at Day 5 Prior to Treatment Among NHPs Treated With 150 mg/kg REGN-EB3



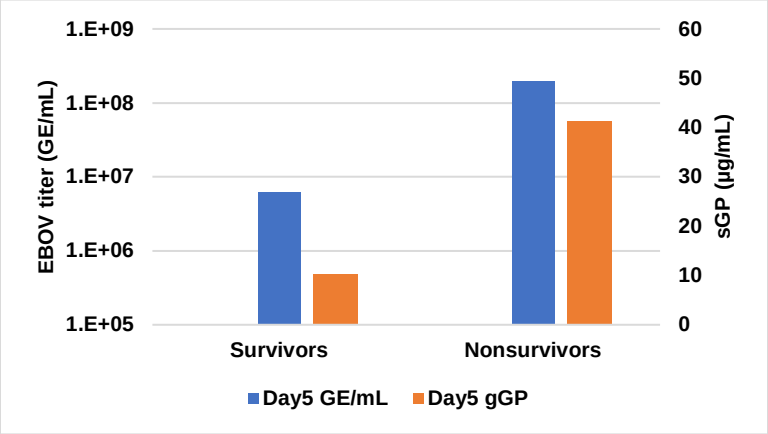
Source: DAV analysis
Y-axis is GE/mL
Abbreviations: EBOV, *Zaire ebolavirus;* NHP, nonhuman primates

Secreted GP concentrations were also determined for nine NHPs treated with 150 mg/kg, six of which survived EBOV challenge and three that died. For the survivors, the mean Day 5 titer and sGP concentrations were 6.2×10^6 GE/mL and 10.3 µg/mL, respectively, compared to 1.8×10^8 GE/mL and 41.3 µg/mL, respectively, for the nonsurvivors (Figure 55).

These results indicate that a higher dose may be more effective for NHPs with baseline EBOV titers $>1\times10^8$ GE/mL and sGP concentrations >10.3 µg/mL. One study tested a dose of 300 mg/kg in NHPs 5 days after challenge and all survived, but of the five NHPs in that study only one had a Day 5 titer of 1.6×10^8 GE/mL and an sGP concentration of

13.6 µg/mL. The variability of the NHP model will make it difficult to assess higher doses.

Figure 55. Mean Day 5 Titers and sGP Concentrations of NHPs That Received 150 mg/kg



Source: DAV Analysis
Abbreviations: EBOV, *Zaire ebolavirus;* sGP, secreted GP; NHP, nonhuman primates; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; sGP, secreted glycoprotein

Overall, the studies performed in NHPs using REGN-EB3 provided proof-of-concept that REGN-EB3 has antiviral activity and was the basis for establishing the 150 mg/kg dose in clinical trials in an emergency setting.

Considerations to be addressed in a PMR: None.

18.1.2.5. Resistance Studies in Animals and Bioinformatics Assessment of Epitope Conservation

Three study reports related to resistance assessments for REGn-EB3 are reviewed below.

Title: A Pharmacokinetic/Pharmacodynamic Assessment of REGN3470-3471-3479 in Rhesus Macaque Monkeys Infected with Ebola Virus

Study number: (b) (4) 2018-008-R3479-PM-18140

Reviewer's note: The PK/PD assessment of REGN-EB3 in rhesus macaques infected with EBOV in this study is reviewed under Section III.18.1.2.4. This review focuses on the resistance analyses performed under this study.

Objectives: One of the objectives of the study was to assess for resistance among NHPs that were treated with REGN-EB3 and failed treatment.

Assessment of Virus Variants in NHP Studies

The investigator reported that select serum samples were subjected to deep sequencing following (b) (4) ***Working Instructions WI-80016:*** *Preparation of RNA samples for deep sequencing using Illumina's TruSeq Sample preparation kit and MiSeq instrument*. Samples for deep sequencing were selected based on treatment group and survival status. The following RNA samples were chosen:

1. Liver and spleen from animals that did not survive to scheduled end of study.
2. Serum sample from the day of death for animals that did not survive to scheduled end of study (or sample from the closest blood collection time point if no terminal sample was available).

3. Serum sample from Day 6 for animals that survived to scheduled end of study, because samples from all animals at this time point exhibited a high viral load by qRT-PCR.

The investigator used sequence data to compare allele frequency changes between and among treated and placebo primates with the goal of identifying substitutions that facilitate viral escape from REGN-EB3. The investigator reported a small number of common substitutions (n=13) in mAb-treated samples, with allele frequency changes in the GP gene being more common than in the rest of the viral genome. The investigator identified one substitution, GP E564K, which was below the level of detection in the viral stock, was absent in the serum sample collected on Day 8 (sequencing depth was too low to accurately estimate the substitution frequency; however, none of the 15 reads covering the GP E564K codon contained the substitution), but increased to a high frequency (44.4%) in one treated NHP (37934, 30 mg/kg mAb dose, found dead in cage on Day 12) over the course of 7 days.

The investigator stated that a rapid change in allele frequency was an indicator of selection and that GP E564K increased to 44.4%, 2.5-fold higher than any other common variant. NHP 37934 had two other nonsynonymous mutations that occurred within a 4 bp window of GP_E564K. A7725G caused an N563T substitution and A7728G caused an E564A substitution. Each of these variants occurred at moderate frequency (N563T=10.9%, E564A=5.1%). The Applicant was unable to find a single read from NHP 37934 that contained the G7727A and A7728G mutations, indicating that the GP_E564A and GP_E564K variants arose independently.

Other variants were found in the GP gene among treated individuals but the changes in allele frequencies from the stock sample were less dramatic. The Applicant stated that this does not preclude them from being considered as possible targets of selection.

An independent assessment of the NGS frequency table provided by the Applicant was performed by Clinical Virology. To identify sites potentially associated with resistance, Clinical Virology used the following criteria, ranked in order of importance:

- EBOV GP amino acid substitutions detected at a frequency of ≥0.05 that occurred in two or more animals that received REGN-EB3 treatment and were detected at <0.01 in the nontreatment group.
- EBOV GP amino acid substitutions detected at a frequency of ≥0.05 that occurred in any known REGN mAb-binding region derived from any animal that received REGN-EB3 treatment and detected at <0.01 in the nontreatment group.
- Any EBOV GP amino acid substitutions detected at a frequency of ≥0.25 that occurred in any animal that received REGN-EB3 treatment and detected at <0.01 in the nontreatment group.

In agreement with the Applicant, the only substitution that met any of the criteria listed above was GP_E564K, which was detected at 0.42 and 0.47 in the liver and spleen of NHP 37934 (30 mg/kg treatment group) on Day 12 (this animal died on Day 12). However, an EBOV GP_I544T substitution was detected in 14 of 23 (61%) animals (including 3 animals in the control group) at frequencies of 0.03 to 0.26. I544 is a cell-culture adaptation associated with the challenge strain, indicating that in these animals the polymorphism is reverting to the amino acid found in the original human isolate (Hoffmann et al. 2017; Ruedas et al. 2017; Ueda et al. 2017). Of note, this polymorphism occurs in the maftivimab (REGN3479)-binding region, and its effect on the neutralization of maftivimab (REGN3479) has been evaluated by the Applicant (Figure 56).

Three additional substitutions were identified that did not meet the criteria listed above, but that could potentially be associated with resistance:

- EBOV GP_G528R was detected at 0.15 and 0.16 in Day 12 liver and spleen samples from NHP 37934 (30 mg/kg treatment group)
- EBOV GP_H549R was detected at 0.14, 0.17, and 0.17 in Day 6 liver and spleen samples from NHP 37953 (150 mg/kg treatment group)
- EBOV GP_N563T was detected at 0.14 and 0.07 in Day 12 liver and spleen samples from NHP 37934 (30 mg/kg treatment group)

Positions EBOV GP_G528R and EBOV GP_H549R are proximal to the maftivimab (REGN3479)-binding site, and EBOV GP_N563T is adjacent to the EBOV GP_E563K/A substitution.

Conclusions

Deep-sequencing analysis of GP sequences sampled from various tissues of NHPs that were treated with REGN-EB3 and failed treatment (died as a result of the challenge) identified one substitution that met the resistance criteria discussed above, substitution GP_E564K. This substitution was detected at a frequency 0.42 and 0.47 in the liver and spleen of NHP 37934 (30 mg/kg treatment group) from sample collected on Day 12, at the time of death. Several additional substitutions were detected that were potentially associated with resistance and these substitutions will be included for phenotypic assessments as part of a postmarketing action.

PMR considerations: Conduct a phenotypic study to determine the impact on binding and antiviral activity of REGN-EB3, atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) using lentivirus-based particles pseudotyped with EBOV GP containing the substitutions I274M, W275L, G528R, I544T, H549R, N563T, and E564A.

Study Title: Assessment of Conservation within Regions of Zaire Ebolavirus Glycoprotein Protected by REGN3470, REGN3471, and REGN3479 Binding Using Viral Sequences from Infected Humans and Nonhuman Primates

Study Number: R3479-PH-20005-SR-01V1

Objectives: The objectives of the studies presented in this report were to:

- Characterize the genetic diversity of regions in EBOV GP protected by individual REGN-EB3 mAb binding (characterized by HDX) using publicly available viral genome sequences from EBOV-infected humans.
- Evaluate the potential presence of REGN-EB3 treatment-related viral escape mutants using viral genome sequences from EBOV-infected rhesus macaques.
- Determine the effect of the T544I substitution on binding of the individual REGN-EB3 mAbs to EBOV GP using an in vitro VLP binding assay.

Methods: In a previous study, HDX-MS was performed to identify regions of EBOV GP protected by binding of the individual REGN-EB3 mAbs (R3479-PH-20004). The EBOV GP regions identified in the HDX-MS study were used in the studies presented in this report to evaluate the potential for antigenic drift and REGN-EB3 treatment-related viral escape mutations.

1. The first study presented in this report characterized the genetic diversity of the EBOV GP regions protected by the individual REGN-EB3 mAbs in the HDX-MS

study using 2,543 publicly available EBOV genome sequences submitted to the National Center for Biotechnology Information database from 1976 to 2018.

2. The second study presented in this report evaluated the potential presence of REGN-EB3 treatment-related escape mutants using RNA isolates from an in vivo efficacy study in EBOV-infected rhesus macaques. ([(b) (4)] 2015-002).
3. The third and final study presented in this report evaluated the effect of the EBOV GP T544I substitution on the binding of the individual REGN-EB3 mAbs. Binding of the individual REGN-EB3 mAbs (atoltivimab [REGN3470], odesivimab [REGN3471], and maftivimab [REGN3479]) or an IgG1 isotype control at a range of concentrations (68.3fM to 667nM) on VLPs expressing wild-type EBOV Makona GP or EBOV Makona GP carrying the T544I substitution was detected using an SULFO-TAG antihuman IgG. Binding, measured as light emission from the SULFO-TAG labels, was reported in relative light units (RLU).

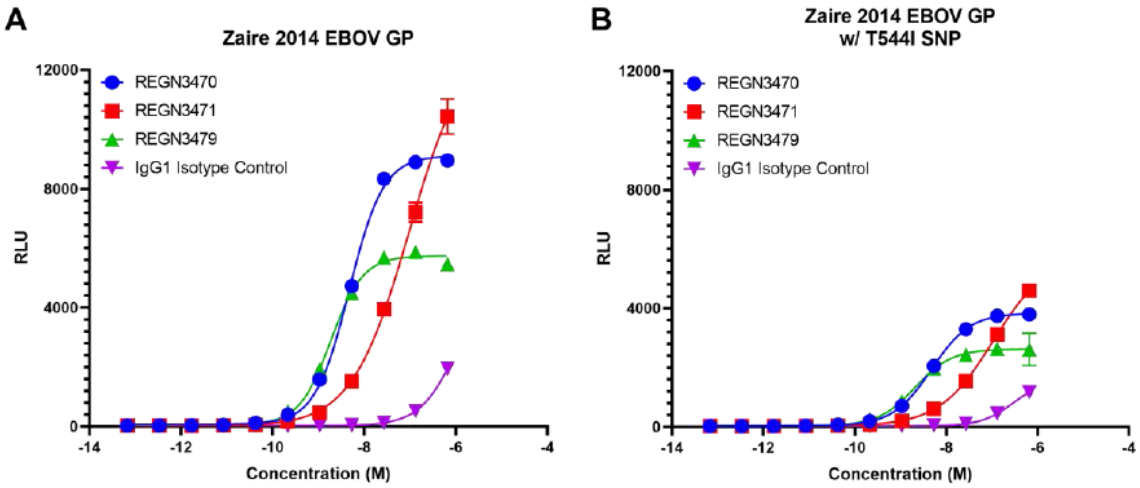
Results

Characterization of the genetic diversity of the EBOV GP regions protected by the individual REGN-EB3 mAbs across EBOV isolates. Sequence analysis of the 2,543 publicly available EBOV isolates revealed a high degree of conservation within the regions of EBOV GP protected by the individual REGN-EB3 mAbs. The most frequent polymorphisms observed in the atoltivimab (REGN3470)- and odesivimab (REGN3471)-protected regions occurred at a frequency of 0.20% (GP_L239S) and 0.34% (GP_D150A), respectively. The most frequent polymorphism in the maftivimab (REGN3479)-protected region, the T544I substitution, was observed at a frequency of 2.0%. However, because this substitution commonly arises during virus propagation in a laboratory setting, it is unclear whether it is associated with circulating EBOV genomes or is the product of tissue-culture passage (Hoffmann et al. 2017; Ruedas et al. 2017; Ueda et al. 2017). Together, these results show that the atoltivimab (REGN3470)-, odesivimab (REGN3471)-, and maftivimab (REGN3479)-binding regions of EBOV GP are highly conserved across EBOV samples.

Sequence analysis of serum viral RNA from saline (placebo) versus REGN-EB3-treated rhesus macaques showed that serum EBOV RNA levels were reduced after REGN-EB3 treatment compared with placebo, indicating that REGN-EB3 was effective in reducing serum EBOV in treated animals. More notably, the analyses did not reveal any treatment-associated enrichment in polymorphisms within the regions of EBOV GP protected by the individual REGN-EB3 mAbs, indicating a lack of detectable REGN-EB3 resistance. The only treatment-emergent substitutions were reported in one NHP in the placebo group (S65P, G496R, and I584L all at ≤5% frequency). However, the T544I cell culture-associated polymorphism was found at a high frequency (≥89%) in baseline (n=12 of 16 NHPs in the study) and posttreatment (n=6 of 16 NHPs in the study) serum samples. Of note, the virus stock used for the inoculation was predominantly I544.

An assay evaluating the ability of the individual REGN-EB3 mAbs to bind VLPs expressing the EBOV GP with the T544I substitution was performed. The binding curves for atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) were similar for VLPs expressing wild-type EBOV GP and EBOV GP with the T544I substitution (Figure 56). The EC_{50} values (when they could be calculated) were similar for both of the EBOV GP variants (Figure 56). Of note, the WT EBOV GP had T544, because it was based on the human EBOV sequence from which the original sample was taken.

Figure 56. The T544I Polymorphism Does Not Affect Binding of the Individual REGN-EB3 mAbs



Source: Figure 1, page 14, Study Number R3479-PH-20005-SR-01V1
Binding of the individual REGN-EB3 mAbs (REGN3470, REGN3471, and REGN3479) or an IgG1 isotype control at a range of concentrations (68.3fM to 667nM) on VLPs expressing (A) wild-type EBOV Makona GP or (B) EBOV Makona GP carrying the T544I substitution was detected using an SULFO-TAG antihuman IgG. Binding, measured as light emission from the SULFO-TAG labels, was reported in relative light units (RLU). Data are from an assay performed in duplicate wells and are plotted as means±SD.
Abbreviations: EBOV, *Zaire ebolavirus;* GP, glycoprotein; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; RLU, relative light units; SNP, single-nucleotide polymorphism

The lower maximum RLU values observed with VLPs expressing EBOV GP with the T544I substitution may be due to lower incorporation into VLPs. The similar EC_{50} values and similar rank-order binding of the three mAbs indicate that the T544I substitution does not reduce the binding ability of any of the individual REGN-EB3 mAbs.

Table 113. Summary of EC_{50} Values for Binding of Individual REGN-EB3 mAbs

Antibody	EBOV Makona GP EC_{50} (M)	EBOV Makona GP w/ T544I EC_{50} (M)
REGN3470	4.7E-09	4.7E-09
REGN3471	NC	NC
REGN3479	1.9E-09	2.1E-09
IgG1 Isotype Control	NC	NC

Source: Table 3, page 14, Study Number R3479-PH-20005-SR-01V1
NC: An EC_{50} value could not be calculated because ant body binding did not reach saturation within the tested range of concentrations
Abbreviations: EC_{50}, half maximal effective concentration; GP, glycoprotein; mAbs, monoclonal antibodies; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab

Conclusions

The results in this study report show that the atoltivimab (REGN3470)-, odesivimab (REGN3471)-, and maftivimab (REGN3479)-binding regions of EBOV GP are highly conserved across EBOV isolates in public databases. Also, in one NHP study detectable treatment-related resistance against REGN-EB3 was not detected in treated animals. In addition, the T544I polymorphism does not affect the neutralization activity of atoltivimab (REGN3470) and maftivimab (REGN3479) in VLPs pseudotyped with EBOV Makona carrying the T544I substitution.

PMR/PMC considerations: None.

Study Title: Assessment of REGN3470, REGN3471, and REGN3479 Neutralization Activities Against Virus-Like Particles Pseudotyped With the E280G or E564K Variant of EBOV GP

Study Report: R3479-PH-20050-SR-01V1

Objectives: The objective of this study was to assess the neutralization activities of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479), as individual mAbs and as REGN-EB3 against VLPs pseudotyped with wild-type EBOV GP, EBOV GP carrying an E280G substitution, or EBOV GP carrying an E564K substitution.

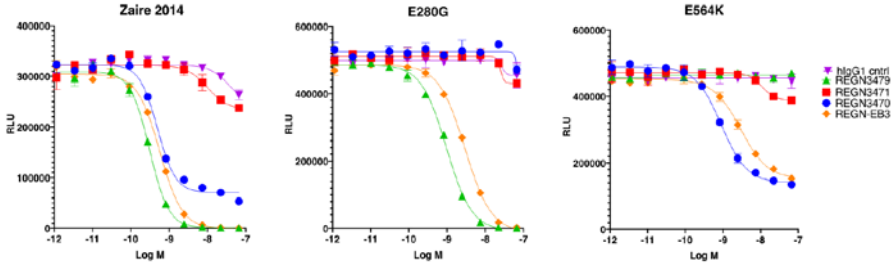
Experimental methods: EBOV GP VLPs were generated by cotransfecting HEK293T cells with a mix of plasmid constructs expressing EBOV Makona GP (wild-type, E280G variant, or E564K variant), human immunodeficiency virus (HIV) Gag-Pol, and an HIV proviral vector encoding firefly luciferase. VLPs were preincubated for 1 hour with atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479), the REGN-EB3 mAb cocktail, or an IgG1 isotype control. VLPs were then added to Huh7 cells for a 72-hour incubation. VLP infection efficiency was detected as luciferase activity and measured as relative light units (RLU). Serial dilutions of antibody (1pM to 0.1µM) were incubated with VLPs for 1 hour at room temperature and added to Huh7 cells.

Origin of Substitutions: An SNP yielding the E280G substitution was identified in late 2019 during the North Kivu EBOV outbreak. The E280 residue is located within the atoltivimab (REGN3470) mAb footprint on EBOV GP identified in previous studies using HDX-MS (R3479-PH-20004). An SNP yielding the E564K substitution was identified in a single EBOV-infected cynomolgus monkey treated with the REGN-EB3 mAb cocktail ([(b) (4)] 2018-008/R3479-PM-18140). The E564 residue is located close to the maftivimab (REGN3479) mAb footprint of EBOV GP (as identified by HDX-MS).

Results

Atoltivimab (REGN3470), maftivimab (REGN3479), and REGN-EB3 neutralized VLPs pseudotyped with wild-type EBOV Makona (described as Zaire 2014 EBOV in the study report) GP; neutralization was concentration-dependent, with EC_{50} values of 0.50nM, 0.31nM, and 0.57nM, respectively (Table 114). Complete neutralization was observed with maftivimab (REGN3479) and REGN-EB3, while only partial neutralization was observed with atoltivimab (REGN3470) (Figure 57). Odesivimab (REGN3471) did not neutralize VLPs pseudotyped with wild-type EBOV GP. Maftivimab (REGN3479) and REGN-EB3 neutralized VLPs pseudotyped with EBOV GP carrying the E280G substitution and neutralization was concentration-dependent, with EC_{50} values of 0.96nM and 0.29nM, respectively (Table 114, Figure 57). Atoltivimab (REGN3470) and odesivimab (REGN3471) did not neutralize VLPs pseudotyped with EBOV Makona GP carrying the E280G substitution.

Figure 57. Effects of GP Sequence Variants on VLP Neutralization



Source: Figure 1, page 10, Study Report R3479-PH-20050-SR-01V1
Abbreviations: REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; RLU, relative light unit; VLP, virus-like particle

Atoltivimab (REGN3470) and REGN-EB3 partially neutralized VLPs pseudotyped with EBOV Makona GP carrying the E564K substitution (identified in EBOV-infected cynomolgus monkeys treated with 30 mg/kg REGN-EB3); neutralization was concentration-dependent, with EC_{50} values of 0.91nM and 0.30nM, respectively. Odesivimab (REGN3471) and maftivimab (REGN3479) did not neutralize VLPs pseudotyped with EBOV Makona GP carrying the E564K substitution.

Table 114. Summary of EC_{50} Values for VLP-Neutralizing Activities of REGN-EB3 mAbs

	EBOV Makona GP Sequence Variants		
Antibody	Wild-type EC_{50} (M)	E280G EC_{50} (M)	E564K EC_{50} (M)
REGN3470	4.97E-10	ND	9.05E-10
REGN3471	ND	ND	ND
REGN3479	3.09E-10	9.59E-10	ND
REGN-EB3	5.69E-10	2.94E-09	2.97E-09
IgG1 Isotype Control	ND	ND	ND

Source: Table 2, page 11, Study Report R3479-PH-20050-SR-01V1
ND: An EC_{50} value could not be determined because concentration-dependent activity was not observed within the tested range of concentrations
Abbreviations: EC_{50}, half maximal effective concentration; mAbs, monoclonal antibodies; REGN3470, atoltivimab; REGN3471, odesivimab; REGN3479, maftivimab; VLP, virus-like particle

The data in Table 114 indicate that there is a >5-fold shift in the REGN-EB3 EC_{50} value in pseudoviruses bearing GP substitutions E280G and E564K in cell culture. Moreover, the E564K GP substitution was derived from an NHP treated with 30 mg/kg of REGN-EB3 and died on Day 12; however, three of the five NHPs survived at this dose level ([(b) (4)] 2018-008-R3479-PM-18140). This observation indicates that resistance to maftivimab (REGN3479) may greatly reduce the activity of REGN-EB3 in vivo.

Conclusions

Two substitutions that impacted susceptibility to REGN-EB3 mAbs were identified:

- Atoltivimab (REGN3470) and odesivimab (REGN3471) did not neutralize VLPs pseudotyped with EBOV Makona GP carrying the E280G substitution.
- Atoltivimab (REGN3470) and REGN-EB3 partially neutralized VLPs pseudotyped with EBOV Makona GP carrying the E564K substitution (identified in EBOV-infected cynomolgus monkeys treated with 30 mg/kg REGN-EB3); neutralization was concentration-dependent, with EC_{50} values of 0.91nM and 0.30nM, respectively. Odesivimab (REGN3471) and maftivimab (REGN3479) did not neutralize VLPs pseudotyped with EBOV Makona GP carrying the E564K substitution.
- There was a >5-fold shift in the REGN-EB3 EC_{50} value in pseudoviruses bearing GP substitutions E280G and E564K in cell culture.
- The E564K GP substitution was derived from an NHP treated with 30 mg/kg of REGN-EB3 and died on Day 12; however, three of the five NHPs survived at this dose level ([(b) (4)] 2018-008-R3479-PM-18140). This observation indicates that resistance to maftivimab (REGN3479) may greatly reduce the activity of REGN-EB3 in vivo.

The observation that the E564K substitution, which negates the neutralization activity of maftivimab (REGN3479) but not REGN-EB3, arose in one NHP that received 30 mg/kg REGN-EB3 and died on Day 12, indicates that resistance to one mAb in the cocktail reduces the antiviral activity of the overall cocktail. This indicates that the three mAbs do not provide overlapping antiviral activity in NHPs.

PMR/PMC considerations: None.

Labeling considerations: Resistance information will be added to the label.

Overall Resistance Conclusions

The Clinical Virology reviewer reviewed the totality of the resistance data provided by the Applicant and concluded that the data provided were insufficient to adequately characterize resistance to the REGN-EB3 cocktail or the individual mAbs therein. The following postmarketing actions will be discussed with the review team and the Applicant:

1. PMR #1: Conduct a phenotypic study to determine the impact on binding and antiviral activity of REGN-EB3, atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) using lentivirus-based particles pseudotyped with EBOV GP containing the substitutions I274M, W275L, G528R, I544T, H549R, N563T, and E564A.
2. PMR #2: Conduct a study to characterize genotypically and phenotypically known atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) resistant variants and those identified in cell culture-based escape studies using VSV-based chimeric virus in binding, neutralization, and ADCC assays with the individual mAbs (atoltivimab [REGN3470], odesivimab [REGN3471], and maftivimab [REGN3479]) and the REGN-EB3 combination. In addition, assess cross-resistance between atoltivimab (REGN3470) and odesivimab (REGN3471) using an ADCC assay.
3. PMC #2: Perform a complete resistance analysis of sequences derived from subjects treated with REGN-EB3 in the PALM trial, if these data become available to you in the future.

19. Other Drug Development Considerations Additional Information

Not applicable

20. Data Integrity-Related Consults (OSI, Other Inspections)

Clinical Inspection Summary

Date	16 September 2020
From	Cheryl Grandinetti, Pharm.D. Clinical Pharmacologist Good Clinical Practice Assessment Branch Division of Clinical Compliance Evaluation Office of Scientific Investigations
To	Alicia Moruf, Pharm.D., RPM Ben Lorenz, M.D., Medical Reviewer Kim Struble, Pharm.D., Medical Team Leader Debra Birnkrant, MD, Division Director, Division of Antivirals (DAV)
BLA #s	761169
Applicant	Regeneron Pharmaceuticals, Inc.
Drug	REGN-EB3 [atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479)]
NME	Yes
Proposed Indication	For the treatment of infection caused by Zaire ebolavirus
Consultation Request Date	14 April 2020
Summary Goal Date	25 September 2020
Action Goal Date	25 September 2020
PDUFA Date	25 October 2020

OVERALL ASSESSMENT OF FINDINGS AND RECOMMENDATIONS

Four Ebola Treatment Units (ETU), Beni, Katwa, Mangina, and Butembo, and the study sponsor, the National Institute of Allergy and Infectious Disease (NIAID), were inspected in support of BLA 761169. The inspections covered one clinical trial, Protocol 19-I-0003, The PAmoja TuLinde Maisha (PALM) study. The study appears to have been conducted adequately, and the study data submitted, including the primary efficacy endpoint data, appear acceptable in support of the respective indication.

BACKGROUND

BLA 761169 was submitted in support of the use of REGN-EB3 for the treatment of Zaire ebolavirus. The key study supporting the applications was the following:

- Protocol 19-I-0003, "A Multicenter, Multi-Outbreak, Randomized, Controlled, Safety and Efficacy Study of Investigational Therapeutics for the Treatment of Patients with Ebola Virus Disease. The PAmoja TuLinde Maisha (PALM) Study"

This was a multicenter, multi-outbreak, randomized, open-label, controlled clinical study, sponsored by the National Institute of Allergy and Infectious Disease (NIAID), evaluating 4 experimental Ebola virus disease therapies, each administered with a backbone of optimized standard of care (e.g., fluid resuscitation, hemodynamic and respiratory support, electrolyte monitoring and replacement, and administration of broad-spectrum antibiotic and antimalarial agents, as indicated). The primary objective of Protocol 19-I-0003 was to compare the mortality at 28 days in patients with Ebola virus disease who received one of three newer investigational drugs (i.e., remdesivir, MAb114, and REGN-EB3) compared to the control arm, ZMapp.

Independent Data Safety Monitoring Board (DSMB) was included to introduce new groups or allow early stopping for futility, efficacy, or safety. The protocol opened as a 3-group trial in November 2018, with REGN-EB3 added as a fourth group in Version 3.0 of the protocol dated 12 Dec 2018. On 09 Aug 2019, the DSMB recommended that patients be assigned only to the MAb114 and REGN-EB3 groups for the remainder of the trial; the recommendation was based on the results of an interim analysis that showed superiority of these groups to ZMapp and remdesivir with respect to mortality.

- *Subjects:* 681 subjects were enrolled
- *Sites***:** 4 Ebola Treatment Units (ETUs) in the Democratic Republic of the Congo
- *Study Initiation and Completion Dates:* 20 Nov 2018 to 11 Oct 2019
- *Database soft lock* occurred on 5 November 2019; *database hard lock* occurred on 17 January 2020

Eligible patients were stratified [by RT-PCR cycle threshold (≤22 vs. >22), Ebola Treatment Center site, and Outbreak] and randomized (in a 1:1:1:1 ratio) to one of the following 4 treatment groups. Group assignments were placed in sequentially numbered envelopes, which were distributed to trial sites and were to be opened sequentially at the time of enrollment.

- Group 1: ZMapp
- Group 2: Remdesivir
- Group 3: mAB114 (ansuvimab)
- Group 4: REGN-EB3 [atoltivimab (REGN3470), odesivimab (REGN3471), maftivimab (REGN3479)]

The total study duration for individual subjects was 58 days (i.e., 30 days following the primary efficacy endpoint of mortality at Day 28). Clinical evaluation (including minimal/optional laboratory assessments) was to be performed within 24 hours of randomization and then on study days 1, 2, 3, 4, 5, 6, 8, 10, 14, and 28. Viral load measurements were collected at admission to the ETU and on study days 1, 2, 3, 4, 5, 6, 8, 10, 14, and 28. Ebola virus quantitative RT-PCR results using the GeneXpert (Cepheid) assay provided both the laboratory diagnosis confirmation of Ebola virus disease and established baseline viral load. Patients who agreed to extended follow-up through Day 58 to help characterize potential late-onset symptoms, evidence of

possible virologic relapse, or other clinical changes, were either seen in person or contacted via phone.

The protocol had defined minimal standards for assessment of efficacy and safety and defined the optimal scheduled assessments for site study personnel to obtain, if the site was able, for the purpose of full longitudinal data collection. However, the inability of a site to collect the full optimal frequency of assessments due to unavoidable resource limitations, and despite best efforts, did not constitute a protocol deviation.

The *primary efficacy endpoint* was the 28-day mortality rate.

Safety Assessments

Only serious adverse events (SAEs) were systematically collected during the study. Events that were considered SAEs were limited to SAEs that were not related to underlying Ebola virus disease, as determined by the investigator, or new or worsening events that were related to the study drug or to a non-Ebola condition, as it was noted that many subjects could enter the study with existing health conditions that meet the SAE criteria.

Paper Source Records

Source document for the study were paper CRFs, informed consent documents, and laboratory reports for safety labs and Ebola PCR results. Data were collected at the ETUs and transcribed onto paper case report forms (CRFs) by the delegated team members at the ETUs. Paper source documents were available for laboratory results (e.g., blood chemistry results as well as the Ebola PCR results). Blood chemistry results as well as the Ebola PCR results were transcribed onto the applicable CRFs by the delegated team members at the sites.

Rationale for Site Selection

All four ETUs, Beni, Katwa, Mangina, and Butembo, and the study sponsor, NIAID, were selected for routine inspection for these applications.

RESULTS (by site):

General Comments

There were 9 clinical investigators who rotated through, staffed, and supervised the conduct of the study for the 4 ETUs. Although only four of the 9 clinical investigators, Drs. Jean-Luc Biampata, Ali Dilu, Isekusu Mpinda Fiston, and Vicky Malengera, were selected to represent the 4 ETUs during the inspections to answer questions, all 9 clinical investigators equally shared oversight of the conduct of the study during their rotation working at their respective ETU.

Furthermore, because of FDA restrictions on conducting inspections in the Democratic Republic of the Congo (DRC), Drs. Biampata, Dilu, Fiston, and Malengera authorized inspections of the 4 ETUs (i.e., Beni, Katwa, Mangina, and Butembo) to be conducted at the NIAID in Bethesda, MD. NIAID provided inspectors access to the PALM Study website (that contained scanned copies of the paper case report forms), the (b) (4) Database (that contained scanned copies of the informed consent documents and GeneXpert source records),and the REDCap electronic data capture (EDC) system used during the conduct of the trial (that contained the case report form data).

Because the NIAID had no documented process in place for providing certified copies (via a validated process or with a dated signature) of the original paper CRFs, study personnel in the DRC and NIAID who performed data entry in the REDCap EDC system, entered data from

scanned CRFs that were not certified. Therefore, during the inspection, FDA field investigators reviewed and verified the study data from these scanned copies of the paper CRFs that were not certified. Please see the NIAID inspection summary below for more information on the process for collecting the study data and scanning, emailing, and uploading scanned copies of the CRFs to the PALM Study website. French translators, provided by NIAID, were present during the inspection.

1. Jean-Luc Biampata, MD

Protocol 19-I-0003

Site: Beni

Boulevard Nyamwisi

Beni, Nord Kivu, Congo

Inspection Dates: 10 – 14 August 2020

At this site for Protocol 19-I-0003, 337 subjects were screened, 335 were randomized [REGN-EB3 (n=72), ZMapp (n=84), MAb114 (n=89), and remdesivir (n=90)], and 196 subjects completed the study (i.e., survived to Day 58). Records reviewed included, but were not limited to, the study protocol and amendments; ethics committee submissions, approvals, and correspondence; subject eligibility criteria; informed consent process and forms; scanned copies of the paper source records; electronic case report forms; primary efficacy endpoint data (i.e., survival status); adverse event reporting; protocol deviations; documentation practices; and monitor logs and follow-up letters. A complete audit of the study records for 30 of the 337 subjects who were screened was conducted.

There was no evidence of under-reporting of adverse events. Survival status (i.e., obtained from scanned copies of discharge and death CRF paper source records) used to support the primary efficacy endpoint was reviewed and verified against the data listings provided by NIAID sponsor for the 156 subjects who were randomized to REGN-EB3 (n=72) and ZMapp (n=84). Survival status for the 90 subjects randomized to remdesivir was not reviewed. No discrepancies were noted.

Issues related to poor documentation were noted during inspection.

a) Subject (b) (6) (randomized to remdesivir) was a neonate born on (b) (6) and was screened and enrolled on (b) (6). No documentation or information was available on the mother's Ebola RT-PCR status.

Reviewer's comment: *Dr. Biampata verbally stated during the inspection that the mother was positive and that she had died in the community. The community response coordinator brought the neonate to the Beni ETU.*

b) For this site, the GeneXpert testing result source records for screening and/or the first negative PCR could not be verified for the following 23 subjects because they were missing: (b) (6) .

Reviewer's comment: *While all GeneXpert testing result source records should have been retained per FDA regulations, the missing source records likely do not impact the reliability of the primary efficacy endpoint data, which was the 28-day mortality rate. These missing source documents were discussed with Dr. Biampata and NIAID. NIAID stated that the missing source*

records were attributed to incomplete file upload to the (b) (4) *database due to internet or to computers in the DRC that had malfunctioned or had been returned to donors. There was no documentation available regarding any corrective and preventative action (CAPA) that was taken. These missing source documents were previously reported to FDA by the Applicant, Regeneron.*

1. Ali Dilu, MD

Protocol 19-I-0003

Site Number: Katwa

Quartier Katwa, Commune Musosa

Katwa, Nord Kivu, Congo

Inspection Dates: 10 – 14 August 2020

At this site for Protocol 19-I-0003, 46 subjects were screened, 46 were randomized [REGN-EB3 (n=10), ZMapp (n=12), MAb114 (n=12), and remdesivir (n=12)], and 27 subjects completed the study (i.e., survived to Day 58). Records reviewed included, but were not limited to, study protocol and amendments, ethics committee submissions, approvals, and correspondence, subject eligibility criteria, informed consent process and forms, scanned copies of the paper source records, electronic case report forms, primary efficacy endpoint data (i.e., survival status), adverse event reporting, protocol deviations, documentation practices, and monitor logs and follow-up letters. A complete audit of the study records for 24 of the 46 subjects who were screened was conducted.

There was no evidence of under-reporting of adverse events. Survival status (i.e., obtained from scanned copies of discharge and death CRF paper source records) used to support the primary efficacy endpoint was reviewed and verified against the data listings provided by NIAID for the 22 subjects who were randomized to REGN-EB3 (n=10) and ZMapp (n=12). Survival status for the 12 subjects randomized to remdesivir was not reviewed. No discrepancies were noted.

2. Isekusu Mpinda Fiston, MD

Protocol 19-I-0003

Site: Mangina

Quartier Masimbembe, Commune

Mangina, Nord Kivu, Congo

Inspection Dates: 10 – 14 August 2020

At this site for Protocol 19-I-0003, 57 subjects were screened, 57 were randomized [REGN-EB3 (n=14), ZMapp (n=13), MAb114 (n=15), and remdesivir (n=15)] and 14 subjects completed to the study (i.e., survived to Day 58). Records reviewed included, but were not limited to, study protocol and amendments; ethics committee submissions, approvals, and correspondence; subject eligibility criteria; informed consent process and forms; scanned copies of the paper source records; electronic case report forms; primary efficacy endpoint data (i.e., survival status); adverse event reporting; protocol deviations; documentation practices; and monitor logs and follow-up letters. A complete audit of the study records for 26 of the 57 subjects who were screened was conducted.

There was no evidence of under-reporting of adverse events. Survival status (i.e., obtained from scanned copies of discharge and death CRF paper source records) used to support the primary

efficacy endpoint was reviewed and verified against the data listings provided by NIAID for the 27 subjects who were randomized to REGN-EB3 (n=14) and ZMapp (n=13). Survival status for the 15 subjects randomized to remdesivir was not reviewed. No discrepancies were noted.

3. Vicky Malengera, MD

Protocol 19-I-0003

Site Number: Butembo

Quartier Lumumba, C/ Kimeni

Butembo, Nord Kivu, Congo

Inspection Dates: 10 – 14 August 2020

At this site for Protocol 19-I-0003, 244 subjects were screened, 243 were randomized [REGN-EB3 (n=63), ZMapp (n=60), MAb114 (n=60), and remdesivir (n=60)] and 70 subjects completed the study (i.e., survived to Day 58). Records reviewed included, but were not limited to, study protocol and amendments; ethics committee submissions, approvals, and correspondence; subject eligibility criteria; informed consent process and forms, scanned copies of the paper source records; electronic case report forms; primary efficacy endpoint data (i.e., survival status); adverse event reporting; protocol deviations; documentation practices; and monitor logs and follow-up letters. A complete audit of the study records for 35 of the 244 subjects who were screened was conducted.

There was no evidence of under-reporting of adverse events. Survival status (i.e., obtained from scanned copies of discharge and death CRF paper source records) used to support the primary efficacy endpoint was reviewed and verified against the data listings provided by NIAID for the 123 subjects who were randomized to REGN-EB3 (n=63) and ZMapp (n=60). Survival status for the 60 subjects randomized to remdesivir was not reviewed. No discrepancies were noted.

4. National Institute of Allergy and Infectious Diseases (NIAID)

Office of Clinical Research Policy and Regulatory Operations (OCRPRO)

5601 Fishers Lane

Bethesda, MD 20892

Inspection Dates: 10 – 14 August 2020

The inspection of the sponsor, NIAID, focused on the control, oversight, and management of Protocol 19-I-0003. The inspection covered roles and responsibilities, organization and its personnel, registration of studies on clinicaltrials.gov, selection and monitoring of clinical investigators, selection of monitors, monitoring procedures and activities, quality management, adverse event reporting, data collection, handling, and management, record retention, financial disclosure, and test article shipping, accountability and management. Records reviewed during the inspection included vendor agreements and contracts, written standard operating procedures (SOPs), documentation of protocol deviations, validation, training, any other documentation related to the operational use of the electronic systems used in the trial (i.e., REDCap system, the PALM Study website, and the (b) (4) repository), adverse event reporting, drug accountability, and monitoring activities.

NIAID contracted with Leidos Biomedical Research, Inc. for clinical trial management, regulatory documentation, data management (e.g., EDC system management, including

validation, CRF creation, data entry, query generation and resolution), laboratory, clinical supplies, and pharmacovigilance.

NIAID and Leidos Biomedical Research had no formal written SOPs or work instructions in place to describe the process for scanning, emailing, and uploading the CRFs to the PALM Study website. In addition, NIAID was also unable to provide documentation that all parties involved in this process were trained. Because there was no documented process in place for providing certified scanned copies (via a validated process or with a dated signature) of the original paper CRFs, study personnel entered and reconciled the study data in REDCap and FDA field investigators verified the study data from copies of the CRFs that were not certified copies.

Reviewer's comment: *During the inspection, a representative from Leidos Biomedical Research described the undocumented process that study personnel used to scan, email, and upload copies of the CRFs to the PALM Study Website as well as their documented procedure for double data entry (and reconciliation of the data) into the REDCap EDC system. Despite the lack of a written documented and validated process and acknowledging that a process (albeit undocumented) existed for ensuring that all CRFs were scanned, emailed, and uploaded correctly and completely to the PALM Study Website, inspectors had some confidence that scanned copies of the CRFs that were reviewed during the inspection had the same information as the original CRFs.*

FDA field investigators noted during the inspection that some subject data for 28 subjects (subject numbers (b) (6)) were entered into REDCap while it was still in the development mode, and audit trails for these subjects were missing. NIAID explained that data managers failed to move the database into production mode at the start of trial and thus data for these subjects had to be re-entered from the scanned pdfs of the CRFs into REDCap once REDCap had been moved into production mode. Tracking any subsequent changes made to this data in REDCap between the time of initial entry in development mode and reentry in REDCap in production mode was missing.

As part of the root-cause for the missing audit trials, FDA field investigators determined that NIAID and Leidos Biomedical Research did not have any formal written SOPs in place for the operational use of electronic systems, for example, for developing, testing, and validating electronic systems and study specific eCRFs used in the trial and for finalizing and moving an EDC system (i.e., REDCap) from in development mode to in production mode. No formal validation test summary report or user acceptance testing reports were provided for REDCap or the PALM Study website.

Reviewer's comments: *The missing audit trails for initial entry of data for subjects (b) (6) do not appear to have an impact on the integrity and quality of the data because copies of the source paper CRFs and other paper source records (i.e., Ebola PCR results and laboratory results, such as blood chemistry results) were available for inspectors to review. FDA inspectors did not solely rely on any data entered in REDCap when verifying the data listings provided by the Applicant. The lack of written SOPs for the operational use of electronic systems used to capture critical data in the trial was discussed with NIAID during the closeout meeting. NIAID acknowledged the inspection finding and promised improvements for future trials, especially in those trials that may rely solely on electronic source data where missing audit trails would be critical to data integrity assessments.*

There was under-reporting of a serious, unexpected, and suspected adverse reaction (SUSAR) of anaphylaxis and death in Subject (b) (6) (randomized to ZMapp). This death occurred on (b) (6). This SUSAR was promptly reported by the clinical investigator to the sponsor, NIAID; however, NIAID failed to report this SUSAR to FDA as a 7- or 15-day expedited IND safety report.

Reviewer's comment: *NIAID noted during the inspection that the SAE was expected as the Investigator's Brochure, Version 8.0, dated 6 November 2018, states "ZMapp, as with any other mAb treatment, has the potential to cause severe, including fatal, infusion reactions." However, this adverse reaction should have been considered unexpected because it was the first death due to infusion-related anaphylaxis. During inspection, NIAID confirmed with the manufacturers of ZMapp that the SUSAR that occurred in Subject (b) (6) was the first case of infusion-related anaphylaxis and death associated with ZMapp. NIAID reported this SUSAR approximately 1 year later in their 2020 IND Annual Report (with no narrative and assessment being provided in the Annual Report). This isolated event was a discussion item at the end of the inspection.*

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21. Labeling Summary of Considerations and Key Additional Information

USPI Labeling Review

Overview of Major Labeling Changes:

- Information highlighted below are significant changes made to the prescribing information from the Applicant's proposed label submitted on October 4, 2019 for INMAZEB (atoltivimab, maftivimab, and odesivimab-ebgn) with the to-be-approved USPI.
- HIGHLIGHTS and TABLE OF CONTENTS were revised for consistency with the full Prescribing Information.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

- The indications statement was modified to add "including neonates born to a mother who is RT-PCR positive for *Zaire ebolavirus* infection" because the proposed indication in adults and pediatric patients was non-specific for pediatric age group. Refer to Section II.6.4.4 for additional details.
- The following limitations of use was added following precedent with influenza labeling, which has similar Limitation of Use for viral infections that can change over time:
  - "*Zaire ebolavirus* can change over time, and factors such as emergence of resistance, or changes in viral virulence could diminish the clinical benefit of antiviral drugs. Consider available information on drug susceptibility patterns for circulating *Zaire ebolavirus* strains when deciding whether to use INMAZEB."

2 DOSAGE AND ADMINISTRATION

2.1 Recommended Dosage

- (b) (4) Therefore, to minimize medication errors the recommendation dosage was revised (b) (4)

2.2 Preparation and Administration

- Additional details were added to preparation and administration instructions to mitigate potential medication errors by providing clear instructions on how to prepare and administer INMAZEB. Additionally, to aid infusion preparation, the volume conversion of 3 mL/kg was added.
- Table 1 INMAZEB Infusion Volumes and Time by Body Weight, was added to provide clear direction on prepared infusion volume and infusion time based on the patient's

weight down to 0.5 kg due to issues with endotoxin. Refer to Section II.6.4.4 and II.7.7.3 for additional details.

5 WARNINGS AND PRECAUTIONS

5.1 Hypersensitivity Reactions Including Infusion-Associated Events

This warning was revised to add infusion-associated events during and post-infusion with INMAZEB and recommendation to slow or interrupt infusion of INMAZEB if the patient develops any signs of infusion-associated events or other adverse events. Refer to Section I.1 and II.7.7.2 for additional details.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

(b) (4) was deleted to focus the safety findings from the PALM trial. The common adverse reactions, including adverse events that occurred during INMAZEB infusion in ≥10% of adult and pediatric subjects, discontinuation and infusion rate adjustment, and selected laboratory abnormalities sections summarized data from the PALM trial comparing INMAZEB to an investigational control. It was noted that there is additional safety information in 228 subjects (190 adult subjects and 38 pediatric subjects) from the expanded access program but only the safety data from PALM trial is presented in Section 6. Refer to Section II.7.6 for additional details.

7 DRUG INTERACTIONS

7.1 Vaccine Interactions

The following language recommending avoiding concurrent administration of live vaccine during treatment with INMAZEB was added. Refer to Section II.8.2 for additional details.

> "No vaccine-therapeutic interaction studies have been performed in human subjects using INMAZEB. However, because of the potential for INMAZEB to inhibit replication of a live vaccine virus indicated for prevention of *Zaire ebolavirus* infection and possibly reduce the efficacy of the vaccine, avoid the concurrent administration of a live vaccine during treatment with INMAZEB. The interval between live vaccination following initiation of INMAZEB therapy should be in accordance with current vaccination guidelines. The efficacy of INMAZEB among subjects who reported receipt of a recombinant live vaccine prior to their enrollment in the PALM clinical trial was similar to subjects who did not receive a vaccine."

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Summary of high rate of maternal and fetal/neonatal morbidity and mortality associated with underlying maternal *Zaire ebolavirus* infection was added based on PALM trial, expanded access program, and published literature. Refer to Section II.8.4 for additional details.

Clinical Considerations

The following statement, "Treatment should not be withheld due to pregnancy" was added. Refer to Section II.8.4 for additional details.

8.2 Lactation

The following statement, "The effects of local gastrointestinal exposure and limited systemic exposure in the breastfed infant to atoltivimab, maftivimab, or odesivimab are unknown" was added. Refer to Section II.8.4 for additional details.

8.4 Pediatric Use

This section was revised to state the safety and effectiveness of INMAZEB for the treatment of infection caused by *Zaire Ebolavirus* have been established in pediatric patients birth to less than 18 years of age based on data from 39 pediatric subjects, including neonates born to a mother who is RT-PCR positive for *Zaire Ebolavirus* based on the PALM trial. The 28-day mortality and safety in adults and pediatric subjects were similar. Refer to Section II.6.4.4 and II.8.3 for additional details.

12 CLINICAL PHARMACOLOGY

12.4 Microbiology

Labeling changes to this section predominantly were made to add additional details pertaining the mechanism of action and antiviral activity. (b) (4) were removed due to conflicting data in the BLA submission.

Antiviral Activity

The following statements were added, "Atoltivimab and odesivimab did not demonstrate any neutralizing activity in Mayinga, Kikwit, and Makona strains of *Zaire* ebolavirus" and "The EC_{50} values of atoltivimab and odesivimab were 2.9 nM and 1.6 nM, respectively, whereas maftivimab did not exhibit any FcγRIIIa signaling activity at the maximum concentration tested, 40 nM." Refer to Section II.7.7.1 for additional details.

Resistance

The following statement was added, "No clinical data are available on the development of EBOV resistance to INMAZEB. The cell culture development of EBOV resistance to INMAZEB has not been assessed to date. A GP_E280G amino acid substitution identified by routine surveillance in the Democratic Republic of the Congo resulted in a loss of neutralization activity of at least 134-fold mediated by the single human monoclonal antibody atoltivimab in a lentivirus-based pseudovirus system. A GP_E564K substitution identified in an infected NHP PK study resulted in a loss of neutralization activity of at least 215-fold mediated by the single human monoclonal antibody maftivimab in a lentivirus-based pseudovirus system. The clinical significance of these substitutions is unknown." Refer to Section II.7.7.1 for additional details.

Immune Response

The following statement was added, "Interaction studies with recombinant live EBOV vaccines and INMAZEB have not been conducted."

13 NONCLINIAL TOXICOLOGY

13.2 Animal Toxicology and/or Pharmacology

(b) (4) was removed because there were no clinically relevant safety concerns. Refer to Section III.13.1.4 for additional details.

14 CLINICAL STUDIES

- Description of the PALM trial was revised to add:
  - Enrolled subjects had "documented" *Zaire Ebolavirus*, confirmed with RT-PCR
  - Subject demographics was revised to add "neonates born to a mother who is cleared *Zaire ebolavirus* following a course of her assigned investigational medication were also eligible to be enrolled at investigator discretion regarding the likelihood that the neonate was infected."
- Efficacy results were revised present 28-day mortality, (b) (4) . Twenty-eight-day mortality was noted as the pre-specified primary efficacy endpoint in the PALM trial, and the primary analysis population was described. Mortality rates in Table 7 were revised to present efficacy results by pediatric age groups and sex. Refer to Section II for additional details.
- Kaplan-Meier curve was revised to show overall mortality (b) (4). Refer to Section III.16.3 for additional details.
- (b) (4) was removed because the efficacy results were already presented in the main efficacy table. Refer to Section III.16.4 for additional details.
- In addition, refer to Section II.6.4 and III.16 for additional details on efficacy results.

22. Postmarketing Requirements and Commitments

Below are the agreed upon PMRs (Table 115) and PMCs (Table 116) for this application.

Table 115. Agreed Postmarketing Requirements

PMR	Milestones
1. Conduct a phenotypic study to determine the impact on binding and antiviral activity against REGN-EB3, atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) using lentivirus-based particles pseudotyped with EBOV GP containing these substitutions to determine shifts in susceptibility: I274M, W275L, G528R, I544T, H549R, N563T, and E564A.	Study Completion: 02/2021 Final Report Submission: 05/2021
2. Conduct a study to characterize genotypically/phenotypically known atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479) resistant variants and those identified through VSV-based chimeric virus cell culture escape studies with respect to binding, neutralization, and ADCC assays with the individual mAbs (REGN3470, REGN3471, REGN3479) and the REGN-EB3 mAb combination (INMAZEB). Specifically assess cross-resistance between atoltivimab (REGN3470) and odesivimab (REGN3471) resistant variants using an ADCC assay.	Study/Trial Completion: 09/2022 Final Report Submission: 03/2023

Table 116. Agreed Postmarketing Commitments

PMC	Milestones
3. Submit a final report with complete, unblinded safety data for all subjects who were enrolled after interim results of the initial phase of the PALM trial and were treated with atoltivimab, maftivimab, and odesivimab-ebgn (REGN-EB3) for *Zaire ebolavirus* infection during the PALM Extension Phase.	Final Report Submission: 03/2022
4. Define the precise epitopes of atoltivimab (REGN3470), odesivimab (REGN3471), and maftivimab (REGN3479).	Study/Trial Completion: 12/2020 Final Report Submission: 04/2021
5. Perform complete resistance analysis of sequences derived from subjects treated with REGN-EB3 in the PALM trial, if access to the data becomes available.	Final Report Submission: 09/2022
6. Collaborate with US public health agencies, other public health agencies and local health authorities, as appropriate to design and conduct a trial to evaluate the efficacy, safety and pharmacokinetics of a higher dose of INMAZEB (atoltivimab, maftivimab, and odesivimab-ebgn) vs. INMAZEB 150 mg/kg in *Zaire ebolavirus*-infected adult and pediatric patients with cycle threshold (CT) values for nucleoprotein targets of less than or equal to 22 to determine if a change in dosing regimen is needed in these patients.	Draft Protocol Submission: 05/2021 Final Protocol Submission: To be determined when such trial is feasible
7. Re-evaluate and update REGN-EB3 drug substance (DS) and drug product lot release and stability specifications based on lots manufactured by the (b) (4) DS processes. The corresponding data, the analysis, and updated specifications will be submitted with the PAS for the registration of the (b) (4) commercial manufacturing process.	Final Report Submission: 01/2021
8. Conduct a real-world Transport Qualification study that includes a product quality assessment using REGN-EB3 drug product. The real-world Transport Qualification study results will be submitted in a final report to the BLA.	Final Report Submission: 08/2021
9. Re-evaluate and optimize the manufacture, qualification and stability controls used to ensure the performance of Ebola Virus-Like Particles (VLPs) in the pseudovirus neutralization assays. The re-evaluation, development study results and the final control strategy for Ebola VLPs will be provided in the final report to the BLA.	Final Report Submission: 12/2021
10. Provide microbial hold time data in a microbial challenge study to support the total in-use time (storage and infusion time) of diluted INMAZEB in 5% Dextrose Injection beyond 4-hours at ambient temperature. The study should be conducted for twice the worst-case in-use time and bracketing the drug product concentrations that would be administered to patients. The study should also be representative of the in-use conditions; for example, neonates may be kept at temperatures above 20-25°C during infusion and the higher temperatures should be simulated in the study supporting in-use conditions.	Final Report Submission: 04/2021

23. Financial Disclosure

Table 117. Covered Clinical Studies: 19-I-0003 (PALM RCT)

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 9		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): None		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): None		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: Enter text here. Significant payments of other sorts: Enter text here. Proprietary interest in the product tested held by investigator: Enter text here. Significant equity interest held by investigator: Enter text here. Sponsor of covered study: Enter text here.		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): Enter text here.		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

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25. Review Team Acknowledgments

Table 118. Reviewers of Interdisciplinary Assessment

Role	Names
Regulatory Project Manager	Alicia Moruf, PharmD
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Nonclinical Team Leader (DPT-ID)	Christopher Ellis, PhD
Clinical Virology Reviewer	Eric Donaldson, Ph.D.
Clinical Virology Team Leader	Jules O'Rear, Ph.D.
Office of Clinical Pharmacology Reviewer(s)	Mario Sampson, PharmD
Office of Clinical Pharmacology Team Leader(s)	Su-Young Choi, PharmD, PhD
Clinical Reviewer	Benjamin Lorenz, MD
Clinical Team Leader	Kimberly Struble, PharmD
Statistical Reviewer	Wen Zeng, PhD
Statistical Team Leader	Thamban Valappil, PhD
Associate Director of Labeling	Stacey Min, PharmD
Cross-Disciplinary Team Leader	Kimberly Struble, PharmD
Division Director (DAV)	Debra Birnkrant, MD
Division Director (DPT-ID)	Hanan Ghantous, PhD, DABT
Division Director (OCP/DIDP)	Kellie Reynolds, PharmD
Division Director (OB/DBIV)	Dionne Price, PhD
Office Director (OID)	John Farley, MD, MPH

DAV, Division of Antivirals; DPT-ID, Division of Pharm/Tox for Infectious Diseases; OCP, Office of Clinical Pharmacology; DIDP, Division of Infectious Disease Pharmacology; OB, Office of Biostatistics; DBIV, Division of Biometrics IV; OID, Office of Infectious Diseases

Table 119. Additional Reviewers of Application

Office or Discipline	Names
OPQ/OBP/DBRR1	Milos Dokmanovic, PhD Jens Fricke, PhD Tzanko Stantchev, MD Marjorie A. Shapiro, PhD (Acting TL)
OPQ/OBP/IO	Vicky Borders Hemphill, PharmD
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OSE/DRM	Elizabeth Everhart, MSN, RN, ACNP Naomi Boston, PharmD (TL) Doris Auth, PharmD (Acting Deputy Division Director)
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TL, Team Leader; OPQ, Office of Pharmaceutical Quality; OBP, Office of Biotechnology Products; DBRR 1, Division of Biotechnology Products Research and Review I; IO, Immediate Office; OPMA, Office of Pharmaceutical Manufacturing Assessment; DBM, Division of Biotechnology Manufacturing, OPRO, Office of Program and Regulatory Operations; OPDP, Office of Prescription Drug Promotion; OSI, Office of Scientific Investigations; OSE, Office of Surveillance and Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRM, Division of Risk Management; DPMH, Division of Pediatrics and Maternal Health; OCPP, Office of Clinical Policy and Programs; OC, Office of the Commissioner; OND, Office of New Drugs

Table 120. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Benjamin Lorenz, MD	OID/DAV	☒ Authored 2, 3, 4, 6, 7, 8, 10, 11, 16, 17, 19, 23 ☐ Approved
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Cross-Disciplinary	Kimberly Struble, PharmD	OID/DAV	☒ Authored 1 ES, contributed to 16 and 17 ☒ Approved 2, 3, 4, 6, 7, 8, 10, 11, 16, 17, 19, 23
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Pharmacology/Toxicology	John Dubinion, Ph.D	OID/DPT-ID	☒ Authored 6, 7, 13 ☐ Approved
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Pharmacology/Toxicology	Hanan Ghantous, Ph.D, DABT	OID/DPT-ID	☐ Authored 6, 7, 13 ☒ Approved
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Clinical Virology	Eric Donaldson, Ph.D.	OID/DAV	☒ Authored 5.1, 6.4.2, 6.4.3 (contributed), 6.4.5, 7.7.1, 18 ☐ Approved
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Clinical Virology	Jules O'Rear, Ph.D.	OID/DAV	☒ Authored 6.4.3 (co-author) ☒ Approved 5.1, 6.4.2, 6.4.3, 6.4.5, 7.7.1, 18
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Clinical Pharmacology	Mario Sampson, PharmD	OCP/DIDP	☒ Authored 5, 6.1, 6.4.2, 8.1, 8.2, 14 ☐ Approved
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Statistical	Wen Zeng, PhD	OB/DBIV	☒ Authored 6.2, 6.3, 6.4.1, 16; (contributed) 6.4.2 ☐ Approved
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[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
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