A drug-disease model for predicting survival in an Ebola outbreak

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Abstract
REGN-EB3 (Inmazeb) is a cocktail of three human monoclonal antibodies approved for treatment of Ebola infection. This paper describes development of a mathematical model linking REGN-EB3’s inhibition of Ebola virus to survival in a non-human primate (NHP) model, and translational scaling to predict survival in humans. Pharmacokinetic/pharmacodynamic data from single- and multiple-dose REGN-EB3 studies in infected rhesus macaques were incorporated. Using discrete indirect response models, the antiviral mechanism of action was used as a forcing function to drive the reversal of key Ebola disease hallmarks over time, for example, liver and kidney damage (elevated alanine [ALT] and aspartate aminotransferases [AST], blood urea nitrogen [BUN], and creatinine), and hemorrhage (decreased platelet count). A composite disease characteristic function was introduced to describe disease severity and integrated with the ordinary differential equations estimating the time course of clinical biomarkers. Model simulation results appropriately represented the concentration-dependence of the magnitude and time course of Ebola infection (viral and pathophysiological), including time course of viral load, ALT and AST elevations, platelet count, creatinine, and BUN. The model estimated the observed survival rate in rhesus macaques and the dose of REGN-EB3 required for saturation of the pharmacodynamic effects of viral inhibition, reversal of Ebola pathophysiology, and survival. The model also predicted survival in clinical trials with appropriate scaling to humans. This mathematical investigation demonstrates that drug-disease modeling can be an important translational tool to integrate preclinical data from an NHP model recapitulating disease progression to guide future translation of preclinical data to clinical study design.
INTRODUCTION

Ebola virus disease (EVD) is increasingly recognized as a threat to public health since its emergence in the Democratic Republic of Congo in 1976 and the burden of an Ebola virus disease (EVD) outbreak cannot be ignored. REGN-EB3 is a combination of three fully human monoclonal antibodies (atoltivimab, maftivimab, and odesivimab-ebgn) that demonstrated reduced fatality rate in patients with EVD when combined with standard of care in the PALM clinical trial.

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Ebola virus (EBOV) is recognized as a threat to public health and the burden of an Ebola virus disease (EVD) outbreak cannot be ignored. REGN-EB3 is a combination of three fully human monoclonal antibodies (atoltivimab, maftivimab, and odesivimab-ebgn) that demonstrated reduced fatality rate in patients with EVD when combined with standard of care in the PALM clinical trial.

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Prior to PALM data availability, a mathematical modeling framework was developed based on preclinical data to describe the antiviral effects and exposure response of REGN-EB3 in non-human primates and to predict survival in humans via translational scaling.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
The model predicted survival rates were generally similar with the survival rates observed prospectively in the PALM trial when time to treatment after infection was assumed to be 5 days. Overall, a single, 150 mg/kg dose of REGN-EB3 was seen to offer greatest protection against EBOV. These simulations provide meaningful information regarding the impact of treatment dose as well as time to treatment on the time course of a disease.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
Our model suggests that, with appropriate scaling to humans, drug-disease modeling can be an important translational tool in integrating preclinical data from a relevant animal model to understand disease progression, guide dose selection for future studies and clinical trial design, and predict the probability of survival in preclinical and clinical dose-ranging studies.

Study Highlights
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further supported clinical development of REGN-EB3 as a single-dose therapeutic for acute EBOV infection. In the PALM trial conducted during an EVD outbreak in the DRC, REGN-EB3 plus standard care significantly reduced the usually high fatality rate of EVD compared with ZMapp plus standard care. In October 2020, REGN-EB3 became the first US Food and Drug Administration (FDA)-approved treatment for EBOV infection in adult and pediatric patients.

It is not always possible to gather dose-ranging data from human challenge studies for infectious diseases with high mortality rates before a medicinal product is approved. As such, animal models which recapitulate EVD characteristics in humans are important for interrogating exposure-response and identifying a dose-regimen that is likely to be safe and efficacious in humans. For Ebola, NHP challenge studies conducted at valid maximum containment Biosafety Level (BSL)-4 laboratory facilities, provided valuable dose-ranging data, but these studies are difficult to conduct under BSL-4 conditions and as such not every pertinent experimental scenario can be tested.

In these situations, mathematical modeling could provide a relevant link between the available nonclinical data and understanding the experimental treatments available for many human diseases. Extrapolation of available information from animal studies to predict outcomes in humans require a framework of dynamic mathematical models and a strong link between models and data such that the model-predicted disease dynamics information can be translated to treatment in humans. Additionally, biomarkers that are of clinical utility may be used to represent disease progression in modeling.

A drug-disease model integrating data on viral dynamics, disease pathophysiology, and outcome (e.g., survival), can be complementary to observed preclinical and clinical data, and allows exploration of a hypothesis that can be difficult to test experimentally, especially under BSL-4 conditions where the limited number of infected animals per study become prohibitive to testing multiple hypotheses. Examples of hypotheses that can be explored include the impact of varying time-to-treatment on clinical outcome (e.g., survival) or the effect of various antiviral treatment modalities (e.g., monotherapy vs. combination therapy). The objective of this analysis, which was initiated prior to the availability of data from the PALM RCT, was to develop a computational drug-survival modeling framework to assess and integrate data across different studies and endpoints, and to apply the an in silico model as a translational tool to predict survival in humans infected with EBOV and the impact of treatment on survival. Here, we describe the development of a drug-disease model linking the effects of REGN-EB3 neutralization of the EBOV to survival in a rhesus macaque model and subsequent translational scaling to predict survival in humans with treatment.

METHODS

Animal housing

All primates were placed in a BSL-4 laboratory 8 days before virus inoculation where they were fed and cared for daily and allowed to acclimatize to their new surroundings.

Data collection

Drug concentration and pharmacodynamic (PD) data from preclinical single- and multiple-dose studies of REGN-EB3 in infected rhesus macaques, conducted under Institutional Animal Care and Use Committee (IACUC) approval, as described previously, were used to develop a mathematical modeling framework to predict the drug response in humans and the effect of time to treatment on drug efficacy in infected rhesus macaques. The software platform, MATLAB version 9.4 (R2018A, MathWorks), was used to develop the model and perform all simulations.

Preclinical studies in rhesus macaques infected with the Kikwit Ebola strain were conducted in a blinded manner in a BSL-4 laboratory at the Texas Biomedical Research Institute or the US Army Medical Research Institute of Infectious Diseases. PD data collected from these studies were used to estimate the model parameters. Collectively in these studies, 36 animals were inoculated with the EBOV at day 0 and received a single-dose REGN-EB3 treatment on day 5. Data collected included survival rate, and laboratory parameters, such as AST, ALT, BUN, creatinine, and platelet count measured after single dose administration of REGN-EB3 with four doses (10, 50, 100, and 150 mg/kg total dose of the three antibodies combined), with nine animals per dose (Tables 1 and 2). In a separate PK study, 12 rhesus macaques were inoculated at day 0 (originally 14 animals were included but two died, and thus were excluded from the dataset due to insufficient data availability) and treated from day 5 with different doses and regimens of REGN-EB3 as follows: a single dose of 150 mg/kg, two doses of 50 mg/kg 3 days apart, or three doses of 50 mg/kg every 3 days (as shown in Table 1).
Population PK model

Drug concentration data were also collected in this analysis. The drug concentration data were used to develop the population PK model. A two-compartment model with a linear clearance rate was subsequently developed (SimBiology 5.8 toolbox) to estimate the concentration of REGN-EB3 (Figure S1). The parameters associated with the PD biomarkers (e.g., synthesis rate and degradation rate of AST, ALT, BUN, creatinine, and platelet count) were collected and/or estimated using literature data.\(^{16,18,21-25}\)

**REGN-EB3 serum concentrations in infected rhesus macaque**

Serum samples from infected rhesus macaques were analyzed for total REGN-EB3 (all three mAbs combined) concentrations using an enzyme-linked immunosorbent assay that measured total anti-EBOV human IgG antibodies. The lower limit of quantification was 0.078 μg/ml in neat serum.

**Determination of serum viral titers**

Analysis of viral load in the sera of infected treated and control animals was conducted by plaque assay, with treatment doses determined on the day of challenge (Table 1; Figure 1a).\(^2\) On the day before challenge, Vero E6 cells were plated in 6-well plates; serial dilutions of the challenge material were then incubated on the monolayer for 1 h at 37°C. The monolayer was subsequently covered with a 0.5% agarose (SeaKem ME Agarose; Lonza) solution postincubation. Following a 1-week incubation period at 37°C, the cells were then covered with neutral red

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### Table 1  Design aspects of preclinical PD and PK studies in infected rhesus macaques

| Property                      | Value                  |
|-------------------------------|------------------------|
|                              | PD                     | PK                     |
| Study                         |                        |                        |
| BSL-4 facility                | USAMRIID               | Texas Biomed           |
| Viral route of administration | Intramuscular          | Intramuscular          |
| Target inoculation, pfu       | 1000                   | 1000                   |
| Control group, n              | 4                      | –                      |
| Treated group, n              | 36                     | 14\(^a\)               |
| REGN-EB3 dose, mg/kg          | Single dose: 10, 50, 100, 150 | • Single dose: 150 (n = 4) |
|                               |                        | • Multiple dose: 100 (50, days 0 & 3; n = 5) |
|                               |                        | • Multiple dose: 150 (days 0, 3 & 6; n = 3) |
| Duration, days                | 35                     | 23                     |
| Day of challenge              | 0                      | 0                      |
| Day of first treatment        | 5                      | 5                      |

Abbreviations: BSL, biosafety level; PD, pharmacodynamic; pfu, plaque-forming units; PK, pharmacokinetic; USAMRIID, US Army Medical Research Institute of Infectious Diseases.

\(^a\)Two animals died due to Ebola infection, and their concentration data were excluded from the model mainly due to sparseness.

### Table 2  Survival outcomes reported after REGN-EB3 treatment in the preclinical PD study and the PALM trial\(^4\)

| Study                  | REGN-EB3 dose (mg/kg) | No. of animals with EVD at day 0 | Survival rate after treatment at day 5 |
|------------------------|-----------------------|----------------------------------|---------------------------------------|
| PD study               | Control               | 4                                | 0 (0%)                                |
|                        | 10                    | 9                                | 4 (44%)                               |
|                        | 50                    | 9                                | 7 (79%)                               |
|                        | 100                   | 9                                | 8 (89%)                               |
|                        | 150                   | 9                                | 8 (89%)                               |
| PALM trial             | 150                   | 155                              | 103 (67%)                             |

Abbreviations: EVD, Ebola virus disease; PD, pharmacodynamic.
and were incubated over night at 37°C. The following day, plaques were counted over a light box. This process was repeated to assess animal’s viremia, but using serially diluted serum was serially diluted instead of challenge material.

### Telemetry and BSL-4 considerations

Telemetry devices were surgically implanted in the rhesus macaques to monitor their temperature throughout these drug concentration and PD studies (T2J; Konigsberg...
Instruments). The data were acquired and analyzed digitally using the Notocord-hem Evolution software platform (version 4.3.0.47, Notocord).

**Mathematical model development**

A viral load-drug dynamics model structure-based on target cell limitation (Figure 2a), was developed to assess the viral titer data over time and to the mechanism of action (MOA) of REGN-EB3 in rhesus macaques (Figure S2). To better understand the pharmacology of the drug, the exposure of REGN-EB3 (PK) was linked to important model parameters, such as clearance of infected cells and production rate of virus ($\delta$ and $p$, respectively, from equations 3 and 4 in Supplementary Materials) using a Hill function. In addition to biological plausibility, local sensitivity analysis was conducted to guide selection of these parameters, however, the results are not included in this paper. A semimechanistic modular modeling approach was used to describe the key hallmarks of EVD (i.e., elevated AST, ALT, and BUN, and decreased platelet counts) by connecting each module to viral load dynamics as a driving force for changes in biomarkers (Figure 2b). Using MATLAB version 9.4 (R2018A) as a platform to formulate numerical techniques, a composite disease characteristic function (DCF) was introduced into the model to describe disease severity and integrated with the ordinary differential equations (presented in Supplementary Materials) estimating the time course for all clinical biomarkers, as depicted in Figure 2c. Scatter plots of DCF were calculated using the equation

$$DCF(t) = \sum_{i=1}^{5} Q_i (x_i(t) - x_{\text{base}})$$

where $x_i(t)$ and $x_{\text{base}}$ are observed biomarker values (i.e., ALT, AST, BUN, PLT, and CR) at time $t$ and baseline, respectively. $Q_i$ is weighting coefficient associated with each hallmark of the disease (Supplementary Methods). The time-varying DCF score was converted to probability of survival using the Kaplan–Meier (KM) estimator to assess the probability of survival (this method is often used to measure the fraction of patients’ lifespan for a certain amount of time after treatment) and the Monte Carlo simulation sampling method. The REGN-EB3 survival model structure describes the MOA of REGN-EB3 and the pathophysiology of EVD (Figure S2). To describe the concentration–time profile of REGN-EB3 in infected rhesus macaques’ serum, a two-compartment population PK model with linear clearance was developed (SimBiology 5.8 toolbox) and used to fit the PK data from the infected rhesus macaque from the NHP PK study. The model structure along with its differential equations are presented in Supplementary Materials.

**Model scaling to humans**

The rules applied to translate PK/PD parameters between rhesus macaque monkeys and humans included use of the equation: $Y = p \cdot BW^b$, where $Y$ is the parameter of interest, $p$ the weight independent parameter, and $BW$ is body weight. Typically, $b$ is assumed to be $-\frac{1}{4}$ for first order rate constants and $\frac{3}{4}$ for zero order constants. Drug-related parameters were not transformed, and amplification factor, interindividual variability, residual error, and dimensionless parameters were not scaled. The average metabolic rate of a cell is lower in larger species and the metabolic rate affects the rate at which a cell synthesizes

**FIGURE 2** REGN-EB3 survival model structure developed from (a) a viral load dynamic model structure; (b) a semimechanistic model structure describing the dynamics of AST (as an example for illustration purposes) in the presence of EBOV; and (c) REGN-EB3 mechanism of action and the disease pathophysiology of Ebola. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CR, creatinine; EBOV, Ebola virus; PK, pharmacokinetic; PLT, platelet count.
deoxyribonucleic acid and proteins, and therefore could affect virus replication; viral replication and T-cell proliferation are slower in larger species. The circulation of T-cells through blood, tissues, and the lymphatic system, which function to eliminate the virus, has been suggested to underlie the generating scaling law. Biological parameter values (Table 3) are used for predictability scaling to humans from the literature (in vivo studies) rather than the use of an allometric scaling approach.

A similar immune system response was assumed for both human and NHP, and the same values for rates of virus production and infected cell removal were used in the viral load dynamics model. The virus clearance rate was scaled using a general scaling law with the power of –\( \frac{1}{4} \). Biological parameters in the model were adjusted using data from the literature. From the literature, we learned that clinical end point values in patients infected with EBOV have the same trend as that seen in rhesus macaque models. The peak values for AST, ALT, creatinine, and BUN in fatal cases were generally consistent with those found in infected rhesus macaque models. Therefore, the current DCF has been used as a criterion for survival analysis, which includes AST, ALT, creatinine, BUN, and platelet counts, with different peak values for BUN and creatinine.

Model validation using clinical trial data

Prior to performing this analysis, a single i.v. dose of REGN-EB3 150 mg/kg was selected for evaluation from the Expanded Access Protocol and the PALM RCT. The population PK model developed in infected rhesus macaques was allometrically scaled to humans to estimate exposure to REGN-EB3 in infected humans at the dose of 150 mg/kg to understand how exposure for this dose in infected humans compared to those associated with maximal efficacy in infected rhesus macaques. To compare predicted survival rates using a scaled model and results from the PALM trial, two simulation scenarios were performed where time of treatment after infection was varied. In the first scenario, 500 virtual patients were created and exposed to REGN-EB3 150 mg/kg at day 5 of infection. For the second simulation, the same virtual population was treated after 2–5 days of infection.

RESULTS

Model predictability by viral load

An overlay of model performance and viral load—time profiles of animals treated with a single dose of REGN-EB3 (10, 50, 100, or 150 mg/kg) demonstrated that the developed model effectively describes the therapeutic effect of REGN-EB3 on EVD. As depicted in Figure 3a, viral load in serum peaked at day 5 after infection, which was also when treatment with REGN-EB3 started. The viral load decreased rapidly with higher doses of REGN-EB3 (100 or 150 mg/kg, single dose) compared with lower doses (10 or 50 mg/kg, single dose). In addition to viral load, the model was able to capture the observed disease pathophysiology data, including AST, ALT, and platelet count levels, as shown in Figures S3a–d, and BUN and creatinine levels, as shown in Figures S3a–b. Figures S1a–b summarizes the performance of the population PK model developed through the comparison of model-predicted and observed concentration values of REGN-EB3.

Effect of REGN-EB3 on disease characteristic function

The DCF contains a composite function of disease pathophysiology information at a specific time. The individual effect–time profiles of DCF, which depicts EVD progression, are shown for different groups of animals in Figure 1. The function over time indicates the time course of disease severity in control and treated groups. As shown in Figure 1a, the untreated group (control) had the highest disease scores over time when compared with the treated groups. As the treatment started on day 5, the DCF values calculated based on observed data decreased over time for the treated groups (REGN-EB3 doses 10, 50, and 150 mg/kg; Figures 1b–d). From day 5 to day 15, the highest dose of REGN-EB3 (150 mg/kg) led to a steady decrease in DCF values and did not deviate significantly from the baseline values before inoculation compared with the other groups (Figure 1d). As the DCF value increased, the probability of survival decreased, as seen in the control group (Figure 1a).

Predicted survival in rhesus macaques treated with REGN-EB3

To evaluate the performance of our model in predicting survival rates in animal studies, virtual animals were created using an MCS sampling method. Two hundred virtually sampled rhesus macaques were treated with a single i.v. dose of REGN-EB3 at different doses on day 5. Comparison of observed survival rates from the PD study, 44%, 79%, 89%, and 89% for doses of 10, 50, 100, and 150 mg/kg, respectively, together with the simulation results, demonstrated the capability of the developed model to predict the survival rate for different dosing regimens as
| Property                                                                 | Value from rhesus macaque model | Value from human model | Reference | Assumption                                   |
|-------------------------------------------------------------------------|---------------------------------|------------------------|-----------|---------------------------------------------|
| ALT and AST degradation in rat cell, 1/day                              | 3                               | –                      | Literature | Same for monkey                             |
| AST half-life in human serum, hours                                     | $17 \pm 5$                      | –                      | Literature | Used allometric for monkey                  |
| ALT half-life in human serum, hours                                     | $47 \pm 10$                     | –                      | Literature | Used allometric for monkey                  |
| Liver AST and ALT activity in liver compared with blood                 | $\sim 10$                       | –                      | Literature | Used to estimate liver AST and ALT concentration |
| Human serum AST, U/L                                                    | 10–40                           | –                      | Literature | –                                           |
| Monkey serum ALT, U/L                                                   | $27 \pm 6$                      | –                      | In-house data | –                                           |
| Monkey serum AST, U/L                                                   | $29 \pm 6$                      | –                      | In-house data | –                                           |
| Delay in viral action on liver function, days                           | $\sim 2$–3                      | –                      | In-house data | –                                           |
| Platelet lifespan for mammals, days                                     | 5–10                            | –                      | Literature | –                                           |
| Normal platelet counts for humans, per $\mu l$                         | -                               | $150,000$–$450,000$    | Literature | –                                           |
| Normal platelet counts for monkeys, per $\mu l$                        | 328,000                         | –                      | In-house data | –                                           |
| CR volume of distribution (human), L                                   | -                               | 33–41                  | Literature | Used allometric for monkeys                 |
| CR clearance rate (human), ml/min                                       | -                               | 35                     | Literature | Used allometric for monkeys                 |
| Normal BUN for human, mg/dl                                             | -                               | 7–20                   | Literature | –                                           |
| Normal BUN for monkey, mg/dl                                            | 14.5                            | –                      | In-house data | –                                           |
| $\lambda$, cell/day                                                     | $5 \times 10^5$ (fixed)         | $5 \times 10^5$        | –                      | –                                           |
| $\mu$, 1/day                                                           | 0.001 (fixed)                   | 0.001                  | –                      | –                                           |
| $\beta$, 1/day*log_{10} (GE/ml)                                        | 0.2045 (fixed)                  | 0.2045                 | –                      | –                                           |
| $\tau$, day                                                            | 2 (fixed)                       | 2                      | –                      | –                                           |
| $\delta$, 1/day                                                        | 2.73 (estimated)                | 2.73                   | –                      | –                                           |
| $P$, 1/day                                                             | 0.001 (estimated)               | 0.001                  | –                      | –                                           |
| $\gamma$, 1/day                                                        | 0.17 (estimated)                | 0.17                   | Estimated             | –                                           |
| Population PK parameter estimates of total anti-EBOV human IgG antibodies in infected rhesus macaque monkeys | | | | |
| $CL (\omega)$, ml/kg/day                                               | 8.4 (0.13)                      | –                      | Estimated             | –                                           |
| $V1 (\omega)$, ml/kg                                                   | 47.88 (0.13)                    | –                      | Estimated             | –                                           |
| $V2 (\omega)$, ml/kg                                                   | 52.1 (2.2)                      | –                      | Estimated             | –                                           |
| $Q (\omega)$, ml/kg/day                                                | 23.74 (0.0062)                  | –                      | Estimated             | –                                           |
| DCF parameter values associated with each biomarker                     |                                 |                        |                       |                                             |
| $Q_{\text{ALT}}$                                                       | 8                               | –                      | Estimated             | –                                           |
| $Q_{\text{AST}}$                                                       | 5                               | –                      | Estimated             | –                                           |
| $Q_{\text{BUN}}$                                                       | 10                              | –                      | Estimated             | –                                           |
| $Q_{\text{PLT}}$                                                       | 3                               | –                      | Estimated             | –                                           |
| $Q_{\text{CR}}$                                                        | 9                               | –                      | Estimated             | –                                           |
| Hill function parameter in viral load dynamics                          |                                 |                        |                       |                                             |
| $V_{m1}$, 1/day                                                        | 3.5                             | –                      | Estimated             | –                                           |
| $K_m$                                                                  | 5.2                             | –                      | Estimated             | –                                           |
| $N_1$                                                                  | 5                               | –                      | Estimated             | –                                           |

(Continues)
illustrated in simulated KM curves (Figure 4a). The model adequately estimated the observed survival rate in rhesus macaques and all REGN-EB doses that resulted in saturation of the PD effects of viral inhibition, reversal of Ebola pathophysiology, and survival.

### Predicted survival in humans infected with EBOV

To evaluate the predictability of our model translated from NHPs to humans, we created 500 virtual patients using the scaled model. Comparison of survival rate from the simulation for an REGN-EB3 dose of 150 mg/kg (70%) and observed data in the PALM RCT (67%) shows that the model prediction of survival was consistent with acceptable accuracy (Figure 4b).\(^4\) Concordance with the actual data was dependent on the time to treatment in the simulation scenario. As shown in Figure 4b, the scenario where treatment was started 5 days after infection in the simulated model showed the greatest concordance with the observed data (70% vs. 67%, respectively), whereas starting treatment 2–5 days after symptom onset in the simulated model resulted in higher predicted survival compared with the observed data from the PALM trial (82% vs. 67%, respectively).

### DISCUSSION

This paper describes the development of an integrated model linking EBOV load and relevant disease pathophysiology (including changes in disease characteristic biomarkers, such as ALT, AST, creatinine, and BUN) to predict the outcome of survival in a rhesus macaque infection model. This modeling approach can be used in conjunction with preclinical data, to support antiviral drug development when conducting dose ranging using a human-challenge study is not feasible (e.g., BSL-4 conditions or when a patient population is not accessible). Using this model, we were able to successfully predict survival in NHPs and, with appropriate scaling, to predict survival in humans comparable to that observed in a human clinical trial (PALM trial), with treatment.\(^4\) Although our model was developed based on preclinical NHP data, the ability of the model to predict survival in the clinical setting is significant due to the practical challenges associated with designing a prospective RCT in EVD.

The model was able to capture the dynamics of disease pathophysiology in the presence of treatment. The simulations indicated that higher doses (both 100 and 150 mg/kg) of REGN-EB3 in the animal study resulted in the disease biomarkers returning to baseline levels more rapidly, thereby increasing the probability of survival.
The simulated survival data were generally similar with the actual observed data in the rhesus macaque model. Because the actual viral time course in comparison to controls could not be determined due to the four deaths in the control group, these simulations provided meaningful information regarding the impact of treatment on the time course of the disease.

Concordance of model predictions of human survival was dependent on time to treatment initiation relative to EBOV infection. When the time to treatment was assumed to be 5 days after infection with an i.v. dose of REGN-EB3 150 mg/kg, the predicted survival rate was 70% compared with 66.5% observed in the PALM trial. However, concordance with the observed PALM data was less when the time to treatment was assumed to be 2–5 days after infection, where the predicted survival rate was 82%. These results suggest that earlier administration of REGN-EB3 would have resulted in a higher survival rate. The time to
The ability of the model to predict survival while altering certain variables, such as time to treatment relative to infection, is important given that whereas the PALM trial was a prospective, multicenter, multi-outbreak, randomized, controlled safety and efficacy trial,\(^4\) generation of future RCT data may be difficult due to the extreme circumstances experienced during an epidemic or a pandemic. Reliance on in silico methods to address unanswered questions can be extremely valuable, especially when used in conjunction with animal models to predict survival in humans.

This integrated model has several strengths; first, it can be used to understand the dose response based on differing time to treatment relative to infection as illustrated, and, second, to predict the effectiveness of antiviral therapies (acting on different parts of the viral life cycle) when used in combination. These advantages are especially important when multiple scenarios cannot be tested experimentally (e.g., BSL-4 conditions). This model can also be used in developing other EVD antiviral therapies, which have different mechanisms of action, by testing different hypotheses.

There are also several limitations to these analyses. It may not be possible to fully capture the disease spectrum (e.g., this model did not account for fever/temperature or other biomarkers of the disease progression, such as white blood cell count). Factors that may potentially affect the PKs of REGN-EB3, such as baseline viral titers and baseline soluble glycoproteins levels, were not evaluated in this model. The PK model was developed using semi-sparse PK data from a small number of animals (\(n = 12\)). PD data were also limited; the model only used data from the treated groups to fully characterize the disease, there were challenges acquiring samples from rhesus macaques due to BSL-4 regulations, and full characterization of the natural history of EBOV infection (without treatment) was not possible due to the limited data set (four animals in the control group). The limited number of animals in each treatment group also made it difficult to describe uncertainty around the survival estimates using the model. Another challenge was the lack of human serum levels from REGN-EB3 treated patients due to inability to transport EBOV blood samples for testing. The model did not evaluate the effect of a wide range of patient characteristics, such as sex, ethnicity, and BW, on exposures to the individual mAbs of REGN-EB3 through population PK based simulations. The actual time from infection to treatment in humans is unknown and therefore extrapolation of data from a rhesus macaque model to a human is limited and should be interpreted with caution. Interindividual variability of drug-related parameters was not scaled in this model; however, existing literature reports interindividual

![FIGURE 4](a) Simulated KM curves for REGN-EB3-treated animals (solid lines) showing actual observed survival rate (% provided below each line) and (b) simulated model survival curve for REGN-EB3-treated humans and actual observed curve from REGN-EB3-treated humans in the PALM trial\(^6\) showing steady state survival rates. (a) The simulated KM curves show adequate consistency between observed and estimated survival rates in infected animal studies. (b) The comparison of simulated survival rate in a clinical setting and observed survival rate in the PALM study (green dotted line) indicates the predictability power of the model. In the simulation (purple line), it was assumed that 500 virtual patients received treatment after 2–5 days of infection. The survival rate related to these patients receiving the treatment at day 5 after infection is shown in a black line. The simulation results obtained using a scaled model were generally similar with the observed data. In addition, it indicates that treatment can increase the chances of survival when provided early. KM, Kaplan–Meier.
variation in humans for the parameters of the biomarkers used in this model.\textsuperscript{32-34}

In conclusion, with a clinically relevant animal model of disease, and knowledge of meaningful PD markers and appropriate scaling to humans, drug-disease modeling can be an important translational tool to predict the probability of survival in preclinical dosing studies as well as in clinical trials. Our model proposes a new pathway for integration of data across different studies and end points, and could be used to predict the probability of survival in preclinical dosing studies and to guide dose selection for future investigations where generating RCT data in humans may be challenging. The scaled model can also be used to look at the impact of patient baseline viral load and minimum dose required to rapidly decrease viral load.

**AUTHOR CONTRIBUTIONS**
M.K.T. wrote the manuscript. All authors designed and performed the research and analyzed the data.

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M.K.T., N.H., J.D.D., A.B., C.A.K., J.K., A.T.DiC., and M.A.K. are Regeneron Pharmaceuticals, Inc. employees/stockholders. C.K. has issued patents (U.S. Patent Nos. 10,787,501, 10,954,289, and 10,975,139) and pending patents, which have been licensed and receiving royalties, with Regeneron Pharmaceuticals, Inc. S.S. is an Excision BioTherapeutics employee/stockholder and former Regeneron Pharmaceuticals, Inc. employee and current stockholder. R.R. is an employee of Blueprint Medicines and former Regeneron Pharmaceuticals, Inc. employee.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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