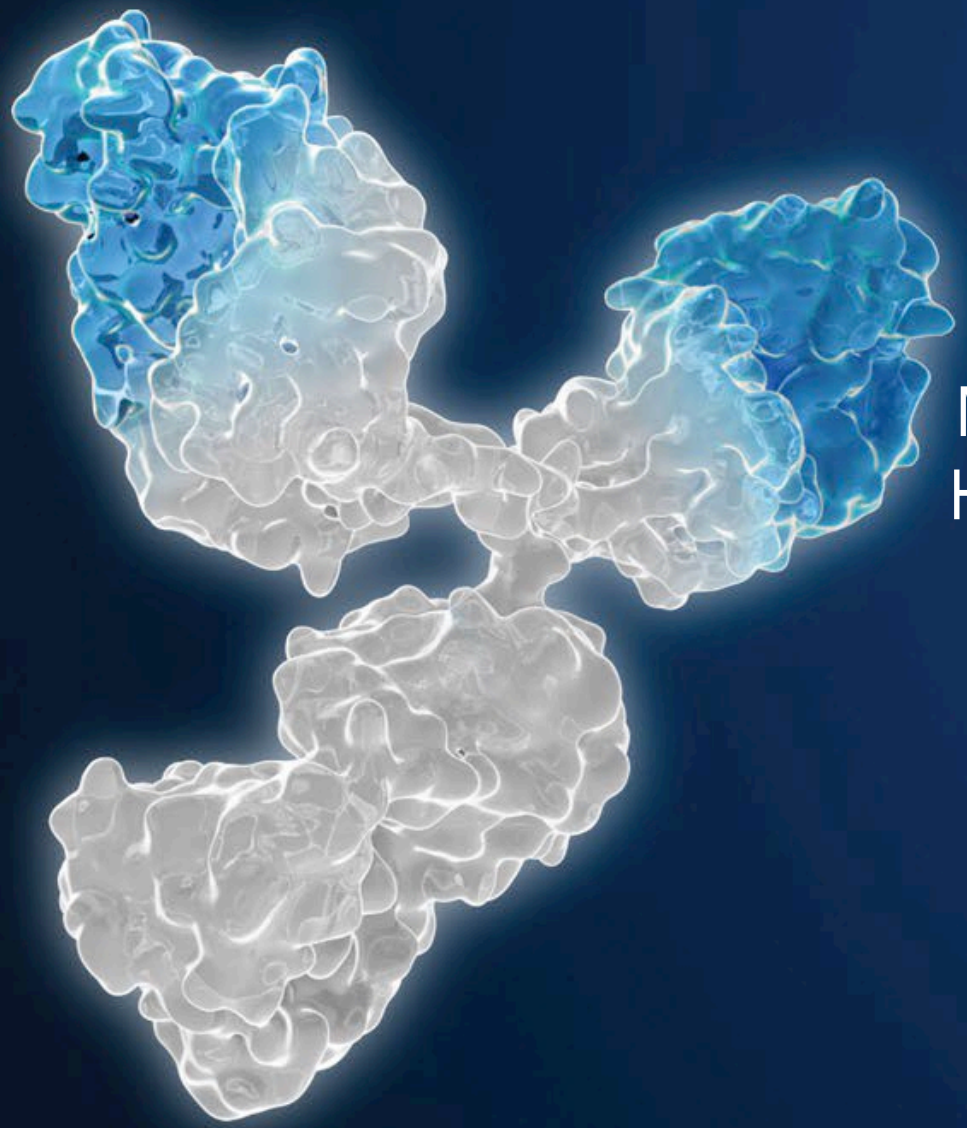

MODELLING AND EXTRAPOLATION TO HUMANS: EBOLA AS A CASE EXAMPLE

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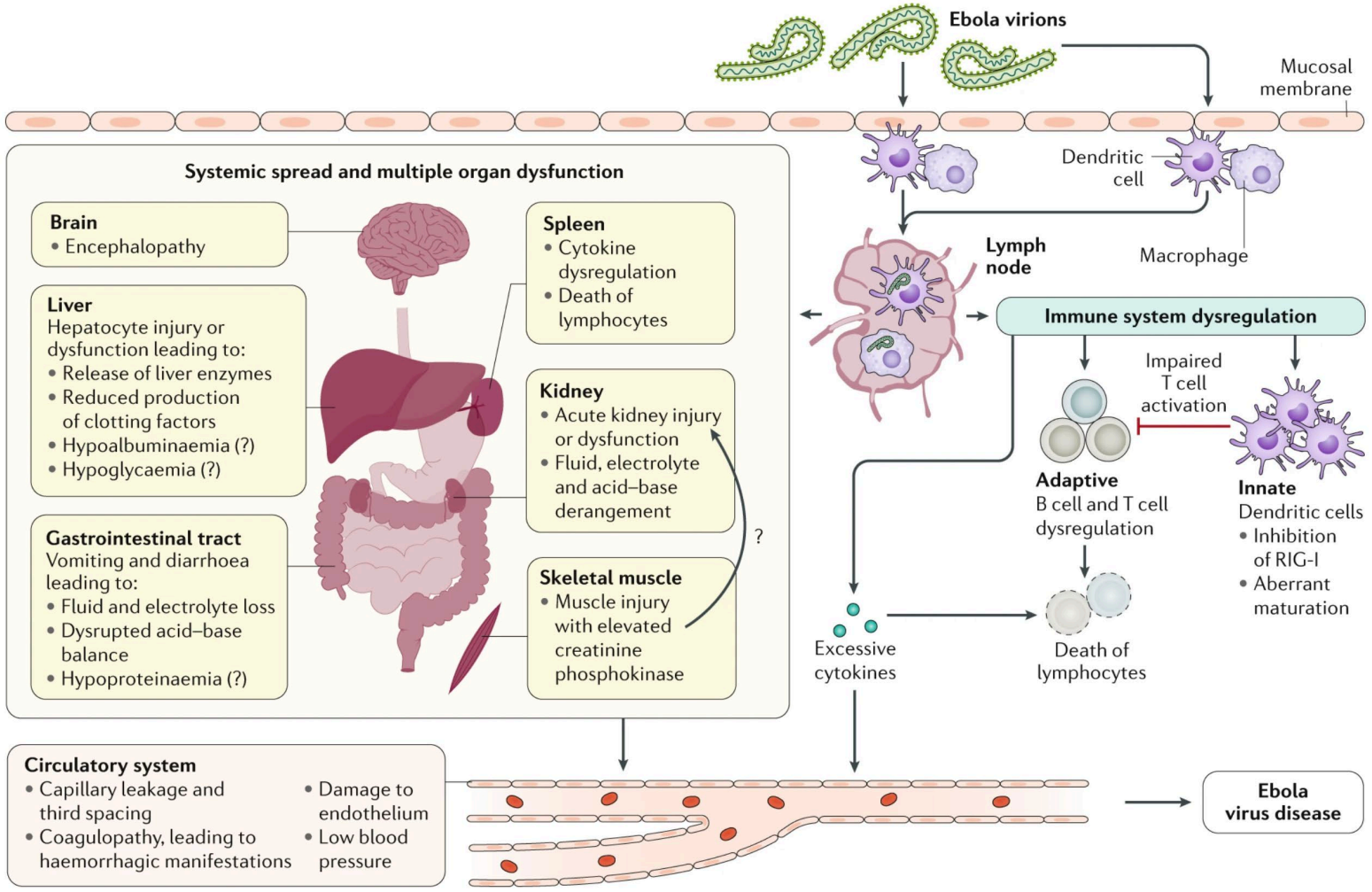
REGENERON
SCIENCE TO MEDICINE®

EBOLA AS A GLOBAL THREAT

- A highly transmissible often fatal infectious disease-also known as hemorrhagic fever
- In August 2018, an outbreak in the Democratic Republic of Congo (DRC) was the second largest that has been recorded since the first description of *Zaire ebolavirus* infection in 1976.
- The World Health Organization (WHO) developed an R&D Blueprint for EVD where the most promising experimental therapeutics would be studied in the context of a randomized, controlled trial.
- Before this paradigm shift, EVD therapies were developed using the animal rule
  - A pathway to allow FDA approval where human efficacy studies are not ethical or feasible and where animal data establish that the drug is likely to produce clinical benefit in humans

PATHOGENESIS OF EBOLA VIRUS DISEASE: DYSREGULATION OF IMMUNE RESPONSE, CYTOKINE STORM, HEMORRHAGIC FEVER, AND MULTIPLE ORGAN FAILURE



Jacob, S.T., Crozier, I., Fischer, W.A. *et al.* Ebola virus disease. *Nat Rev Dis Primers* **6**, 13 (2020).

Inmazeb (EB3) was the first mAb therapeutic FDA approved for treatment of a viral infection. First treatment approved for Ebola.

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 12, 2019 VOL. 381 NO. 24

A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics

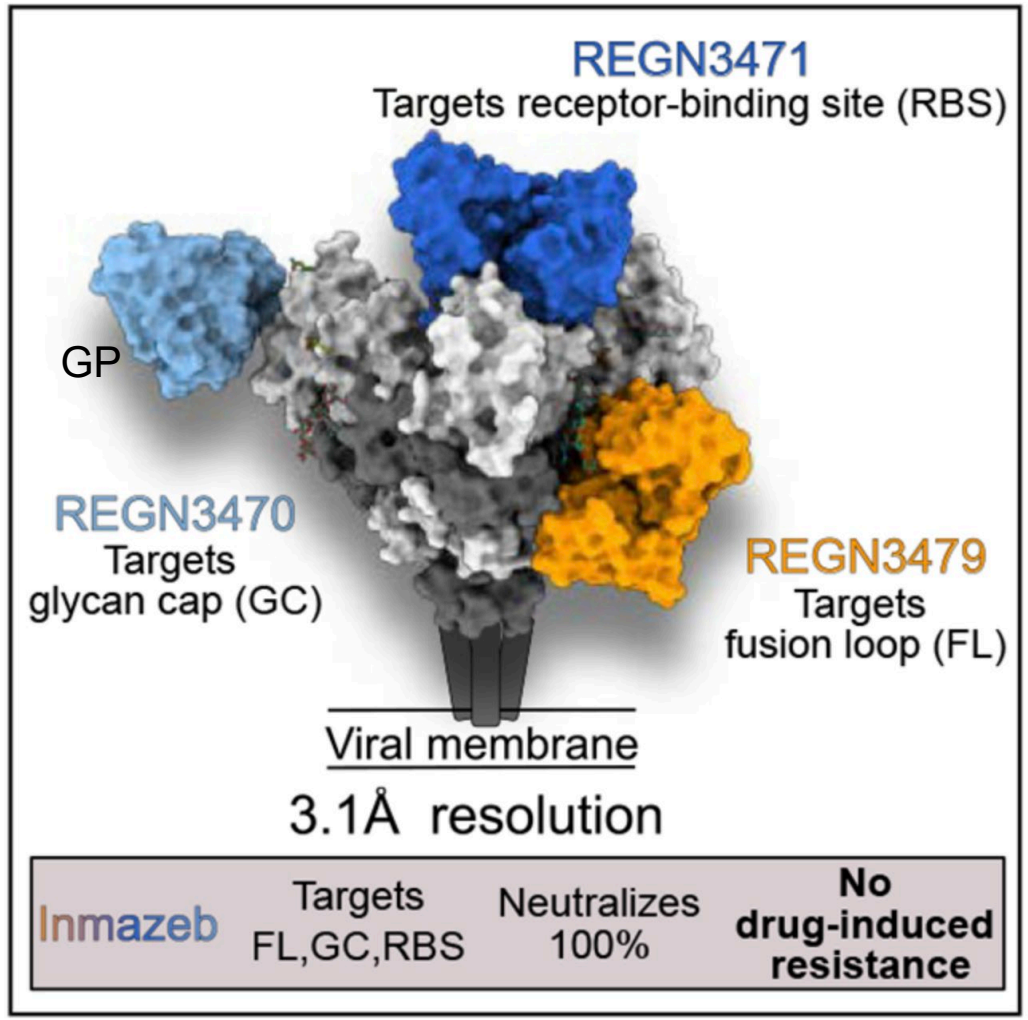
Sabue Mulangu, M.D., Lori E. Dodd, Ph.D., Richard T. Davey, Jr., M.D., Olivier Tshiani Mbaya, M.D., Michael Proschan, Ph.D., Daniel Mukadi, M.D., Mariano Lusakibanza Manzo, Ph.D., Didier Nzolo, M.D., Antoine Tshomba Oloma, M.D., Augustin Ibanda, B.S., Rosine Ali, M.S., Sinaré Coulibaly, M.D., Adam C. Levine, M.D., Rebecca Grais, Ph.D., Janet Diaz, M.D., H. Clifford Lane, M.D., Jean-Jacques Muyembe-Tamfum, M.D., and the PALM Writing Group, for the PALM Consortium Study Team*

Regeneron, in the thick of COVID-19 fight, snags historic FDA approval for Ebola treatment

By **Eric Sagonowsky** • Oct 15, 2020 10:30am

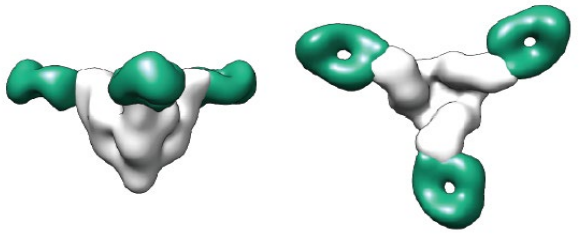
Rayaprolu et al., *Cell Host and Microbe*, 2023

REGN-EB3 (INMAZEB) IS APPROVED FOR TREATMENT OF ZAIRE EBOLAVIRUS.
THE MAB COCKTAIL IS COMPOSED OF THREE NON-COMPETING ANTIBODIES WITH COMPLEMENTARY PROPERTIES.

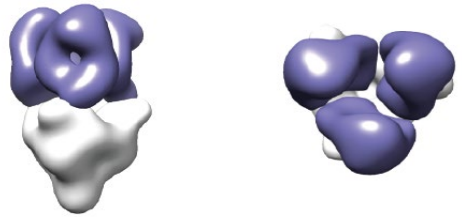


Inmazeb	Targets FL,GC,RBS	Neutralizes 100%	**No drug-induced resistance**

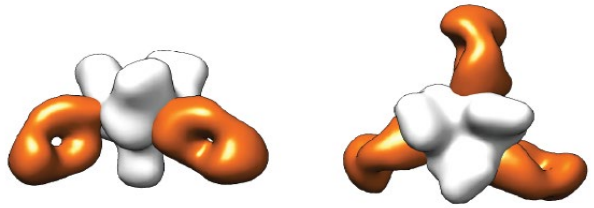
- **REGN3470** Neutralization and Effector Function (ADCC)



- **REGN3471** Effector Function and binds sGP (soluble GP)



- **REGN3479** Neutralization



Pascal et al., *J Infect Dis*, 2018

Rayaprolu et al., *Cell Host and Microbe*, 2023

THE RHESUS-MACAQUE NHP MODEL

- Rhesus Macaque is the gold standard animal model for Ebola virus disease approved by the FDA for use under the Animal Rule
- Recapitulates human EVD: Rhesus macaques develop similar clinical, virologic and pathophysiologic features seen in human EVD
- Similar viral load time course
- Immune system resembles human immune system; both innate and adaptive immunity
- Provides endpoints difficult to achieve in humans
  - Frequent blood sampling
  - Real time organ pathology
  - Serial biopsies
- Endpoints Measured Included:
  - EB3 Concentrations in serum over time
  - AST, ALT, BUN, CR, Platelets and Temperature
  - Survival

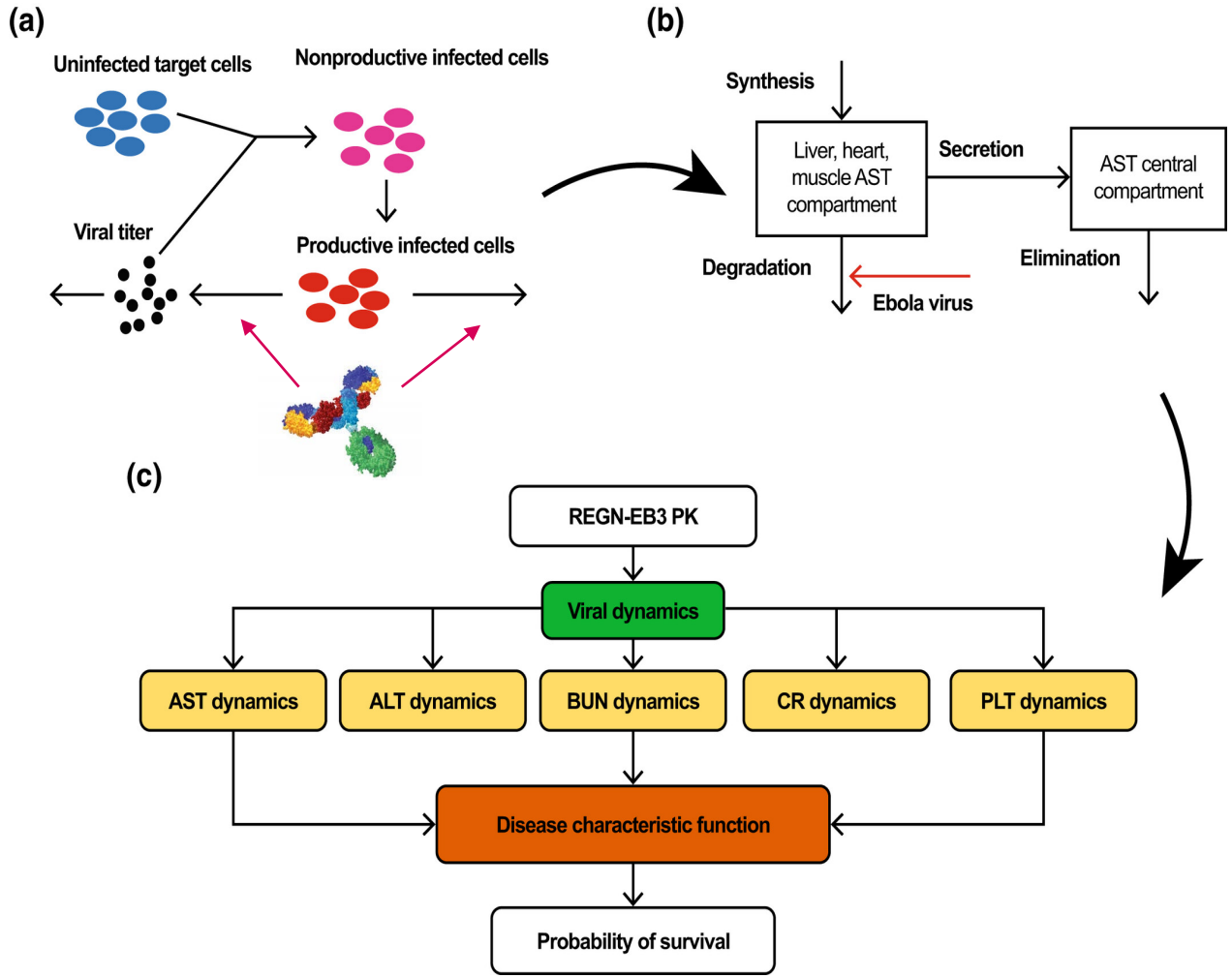
RHESUS MACAQUE DATA AVAILABLE AND THE CHALLENGE OF BSL-4 EXPERIEMNTAL CONDITIONS

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

Property	Value	
Study	**PD**	**PK**
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

Clinical Translational Sci, Volume: 15, Issue: 10, Pages: 2538-2550, First published: 27 July 2022, DOI: (10.1111/cts.13383)

WE CONCEPTUALIZED A MODULAR APPROACH: CONNECTING EB3 PK, EBOLA VIRAL DYNAMICS, EBOLA DISEASE CHARACTERISTICS AND SURVIVAL OUTCOME IN A SINGLE QUANTITATIVE FRAMEWORK



The modules, components

The Integrated framework

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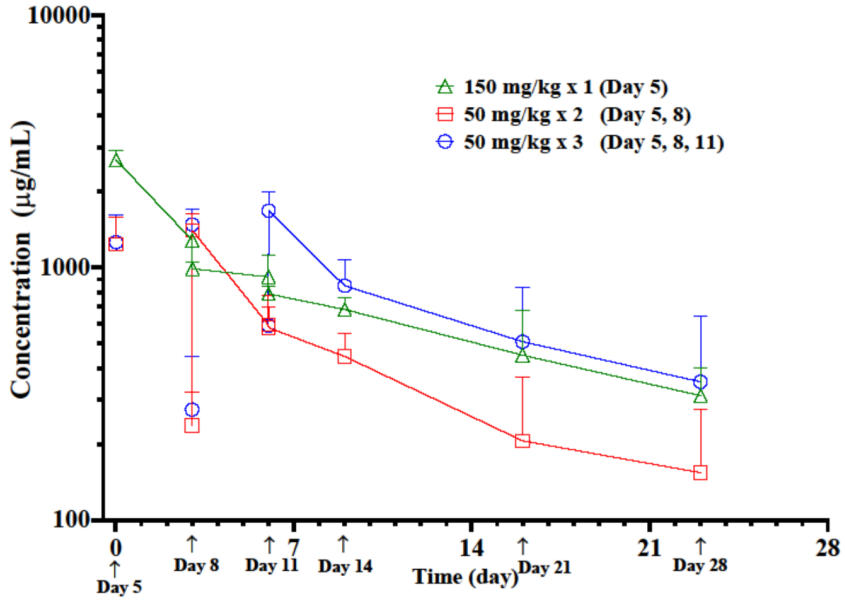
EB3 PHARMACOKINETICS ARE LINEAR EVEN IN THE PRESENCE OF EBOLA INFECTION

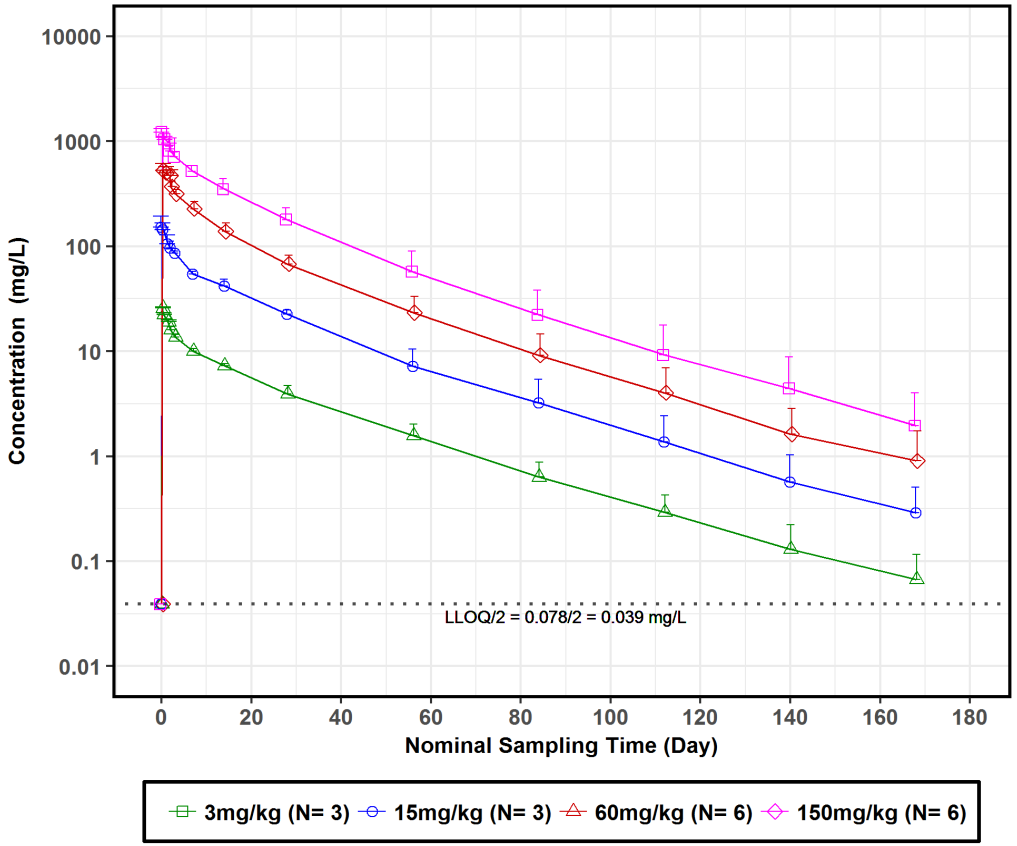
EB3 Concentration Time Profiles in Infected Rhesus Macaques

Figure 2: Serum Concentration over Time Profile of anti-Ebola mAbs Following IV Administration to Infected Rhesus Macaques

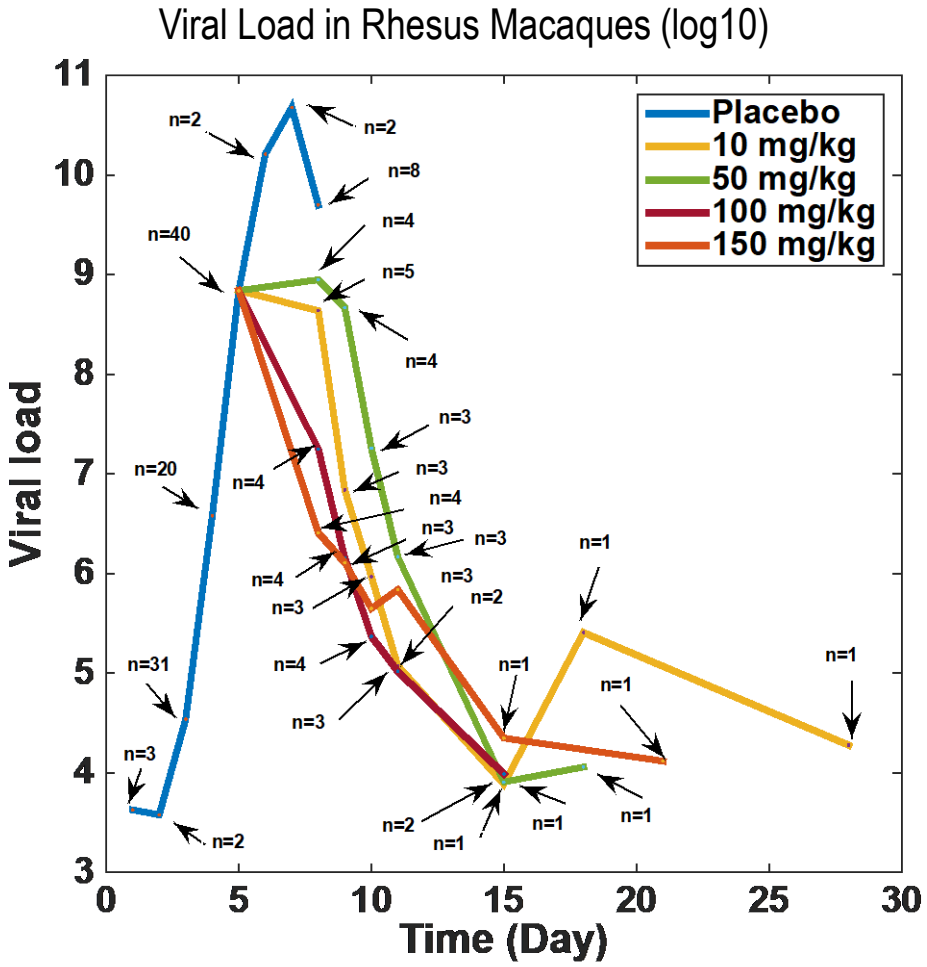


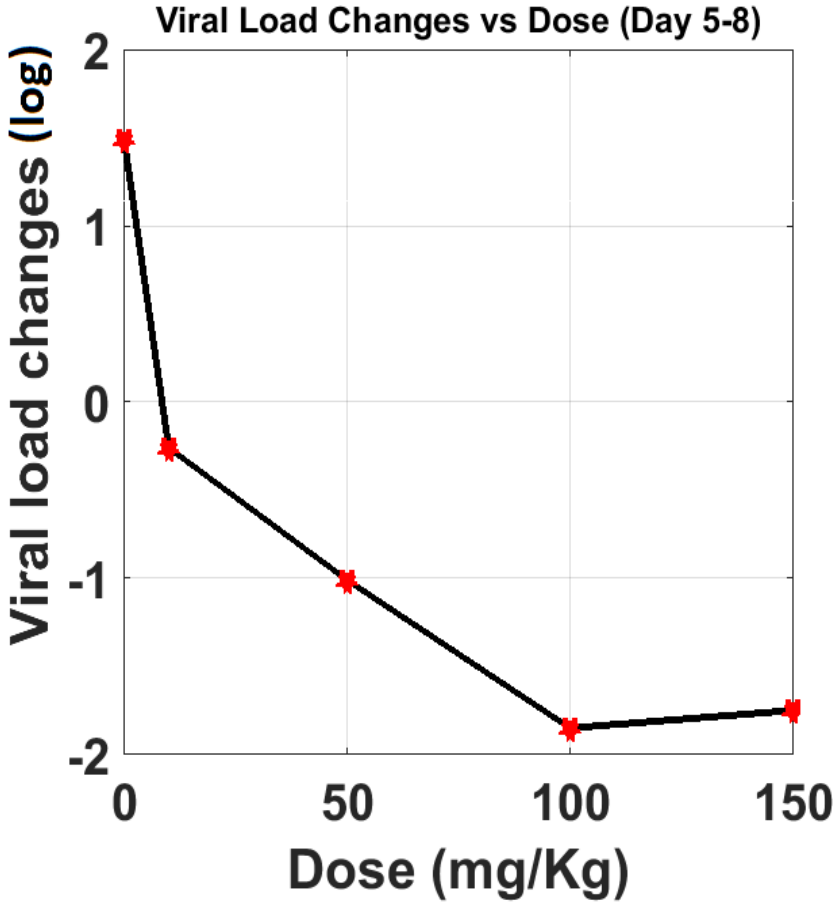
a. Initial dose(s) of anti-Ebola mAbs were given 5 days post-infection with EBOV.
b. Concentrations of total REGN3470, REGN3471, REGN3479, or a combination thereof (total anti-Ebola antibodies) in cynomolgus macaques serum were measured using a validated ELISA for total human IgG with a lower limit of quantitation (LLOQ) of 0.078 µg/mL in neat macaque serum.
c. Exposures are shown post-dose on Day 5, and pre- and 5 minutes post-dose on Day 8 and Day 11.

Concentration Time Profiles in Healthy Human Volunteers



EBOLA VIRAL DYNAMICS- RHESUS MACAQUES CHALLENGE MODEL- DECREASING VIRAL LOAD WITH INCREASING DOSE

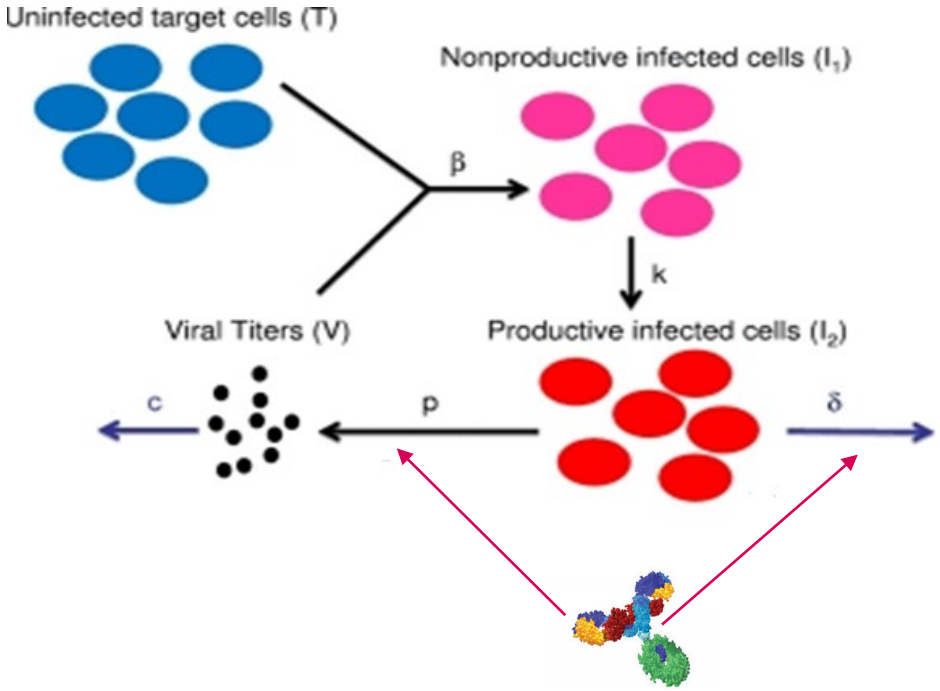




- Viral load peaks at day 5-7
- Viral loads as high as 11 log10
- Peak viral load, a correlate of EVD severity, decreases with dose but plateaus between 100-150 mg/kg .

THE CLASSIC VIRAL DYNAMICS MODEL- INCORPORATING DRUG EFFECT

This model describes the viral titer curve



EB3 Drug Effect: reducing production of free virus and removal of infected cells

EB3 Drug Concentrations as a forcing function on viral dynamics

Rate of change of free virus (V)

$$\frac{dV}{dt} = p\left(1 - \frac{V_{M2}C^{n2}}{K_{M2}^{n2} + C^{n2}}\right)I - \gamma V$$

Rate of change of infected cells (I)

$$\frac{dI}{dt} = \frac{L}{\tau} - \delta\left(1 + \frac{V_{M1}C^{n1}}{K_{M1}^{n1} + C^{n1}}\right)I$$

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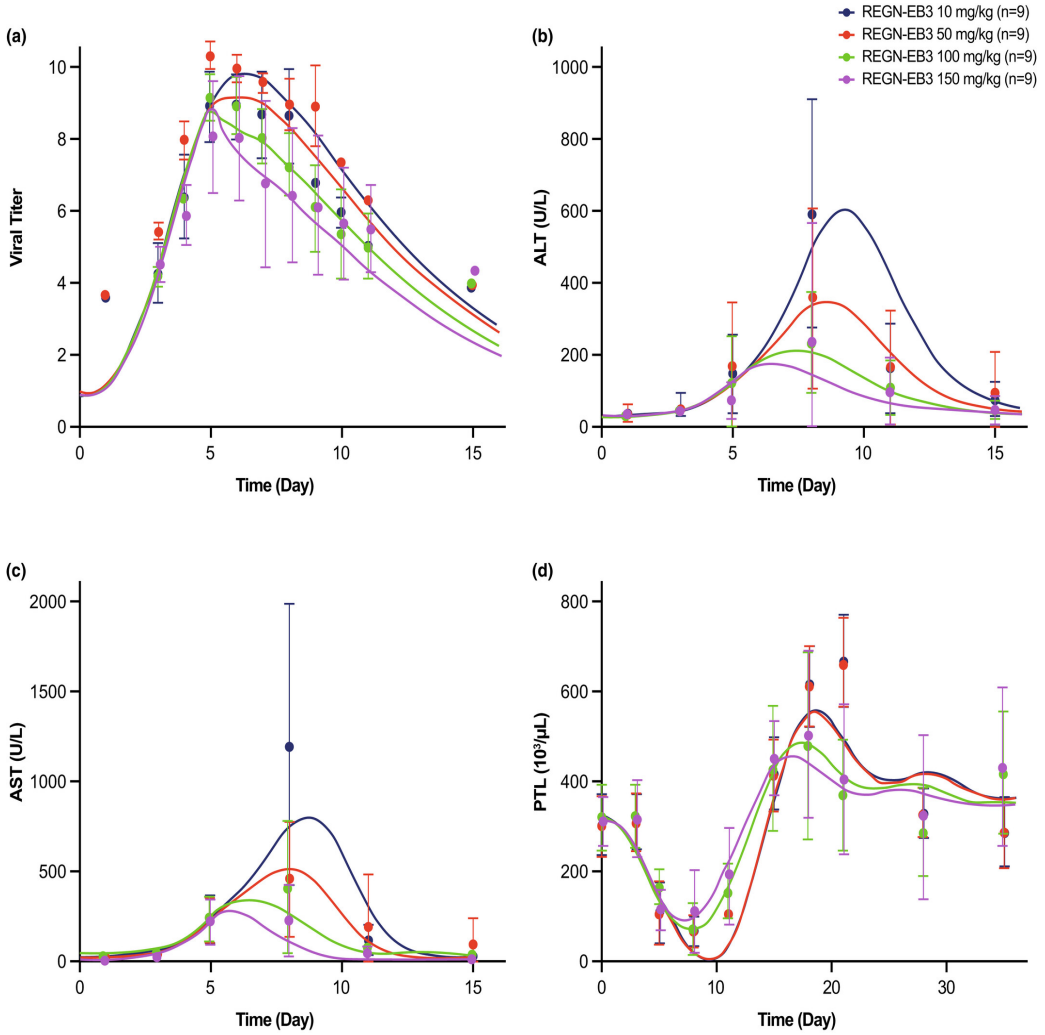
VIRAL LOAD DRIVES EBOLA DISEASE SEVERITY BIOMARKERS (FORCING FUNCTION)



Rate of change of ALT

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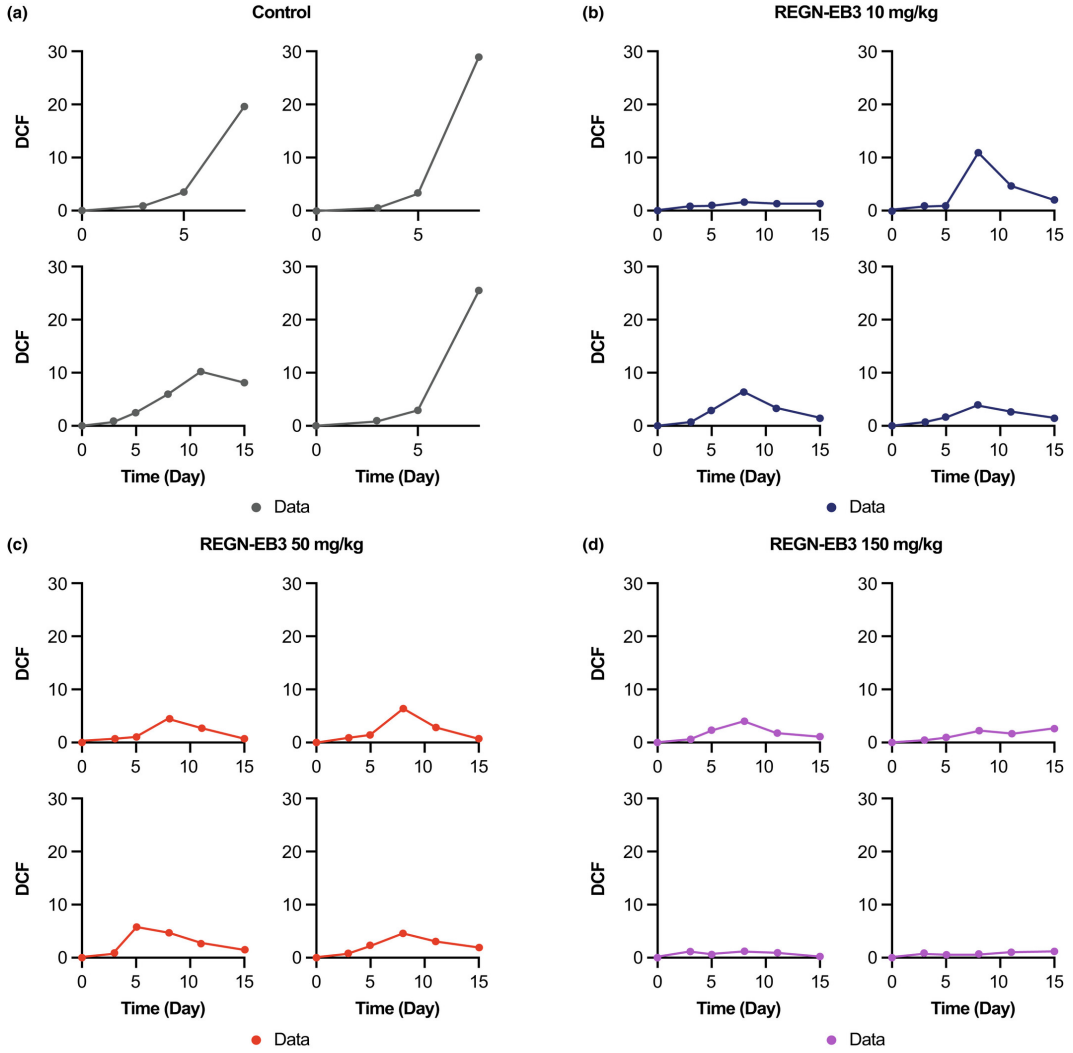
THE DISEASE CHARACTERISTIC FUNCTION (DCF) OVER TIME INDICATED HIGHEST DISEASE SEVERITY FOR THE CONTROL GROUP AND DECREASED WITH INCREASING DOSE OF EB3



- *DCF(t) = $\sum_{t=1}^{5} Qt\ (xi(t) - xbase)$ where xi(t) and xbase were the observed biomarker value (i.e., ALT, AST, BUN, PLT, and CR) at time t and baseline, respectively.*
- *Qi was a weighting coefficient associated with each hallmark of the disease.*
- *Weighting Q was determined by univariate analysis with the biomarker severity most strongly correlated with death receiving a higher weight Q.*
- *DCF was linked to survival by identifying a threshold DCF at which the probability of death was maximized (i.e. DCF=20 in this case)*
- PS(T) = 1- $\frac{\sum dead\ animal\ at\ time\ T}{\sum total\ number\ of\ anima\ l\ at\ risk\ at\ time\ T}$
- *Limitation : we did not use telemetry (body temperature) data*

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SCALING FROM ANIMAL TO HUMANS USED FIRST PRINCIPLES

TABLE 3 Drug-disease model parameters

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 ± 5	–	Literature	Used allometric for monkey
ALT half-life in human serum, hours	47 ± 10	–	Literature	Used allometric for monkey
Liver AST and ALT activity in liver compared with blood	~10	–	Literature	Used to estimate liver AST and ALT concentration
Human serum AST, U/L	10–40	–	Literature	–
Monkey serum ALT, U/L	27 ± 6	–	In-house data	–
Monkey serum AST, U/L	29 ± 6	–	In-house data	–
Delay in viral action on liver function, days	~2–3	–	In-house data	–
Platelet lifespan for mammals, days	5–10	–	Literature	–
Normal platelet counts for humans, per μl	-	150,000–450,000	Literature	–
Normal platelet counts for monkeys, per μl	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	–
λ, cell/day	$5*10^5$ (fixed)	$5*10^5$	–	–
μ, 1/day	0.001 (fixed)	0.001	–	–
ß, 1/day*$\log_{10}$ (GE/ml)	0.2045 (fixed)	0.2045	–	–
τ, day	2 (fixed)	2	–	–
δ, 1/day	2.73 (estimated)	2.73	–	–
P, 1/day	0.001 (estimated)	0.001	–	–
γ, 1/day	0.17 (estimated)	0.17	Estimated	–

The rules applied to translate PK/PD and disease model parameters between rhesus macaque monkeys and humans included :

- *use of* allometric scaling : *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 −1/4. for first order rate constants and 3/4 for zero order constants.*
- *The average metabolic rate of a cell and CL parameters are therefore lower in larger species*
- *Drug-related parameters were not transformed.*
- *Immune system parameters were assumed to be similar*

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OBSERVED SURVIVAL DATA: RHESUS MACAQUES AND HUMANS (PALM TRIAL)

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%)
		No. of patients with EVD at day 1	**Survival rate after treatment at day 28**
PALM trial	150	155	103 (67%)

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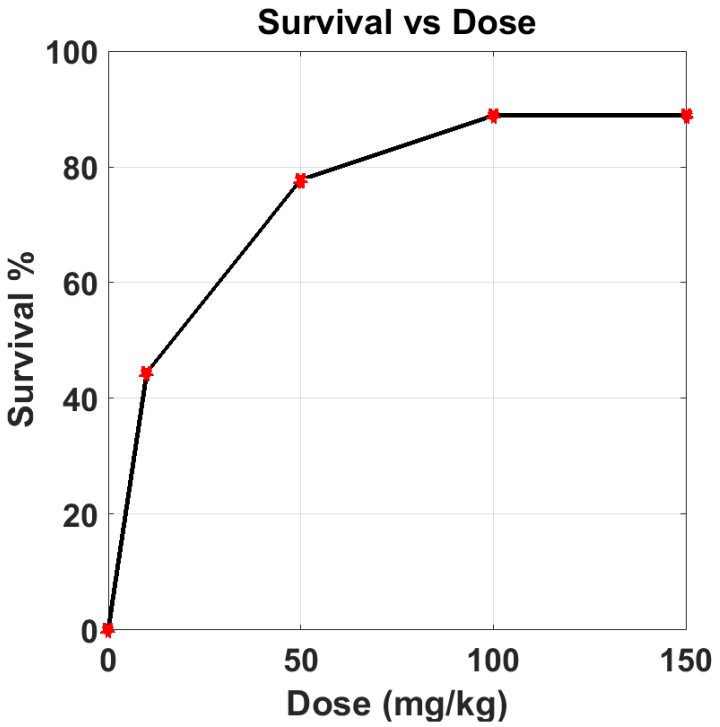
PREDICTING SURVIVAL IN EBOLA INFECTED PATIENTS RECEIVING EB3 THERAPY- EXTRAPOLATION TO PALM TRIAL OUTCOME

Observed Survival versus Dose: Rhesus Macaque

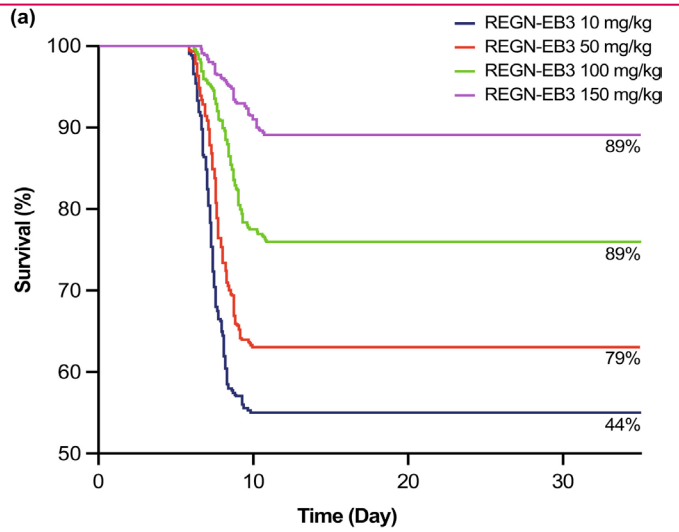


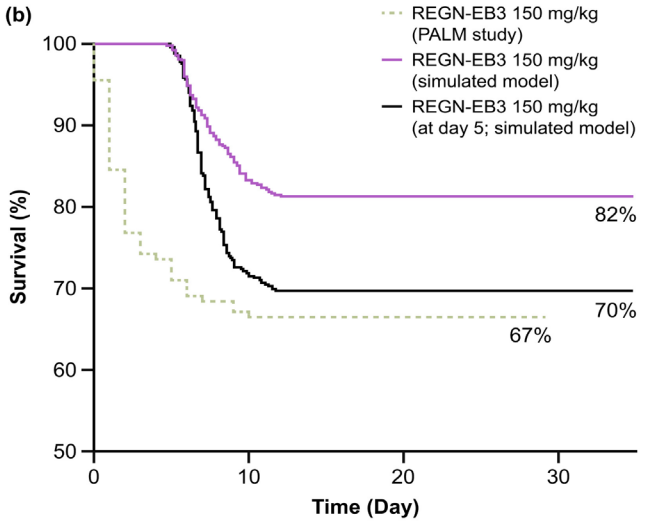
Model Survival Predictions versus observed data

(a) Simulated survival curves (solid lines) and observed survival rate (% provided below each line) by EB3 dose

(b) Simulated model survival curve for EB3- treated humans and actual observed curve from REGN-EB3-treated humans in the PALM trial showing steady state survival rates.

- *Purple line: it was assumed that 500 virtual patients received treatment after 2–5 days of infection (82% survival).*
- *Black line: Patients receiving the treatment at day 5 after infection (70% Survival)*
- *Grey line: Palm Trial Observed Surivival at day 28 (67% Survival)*





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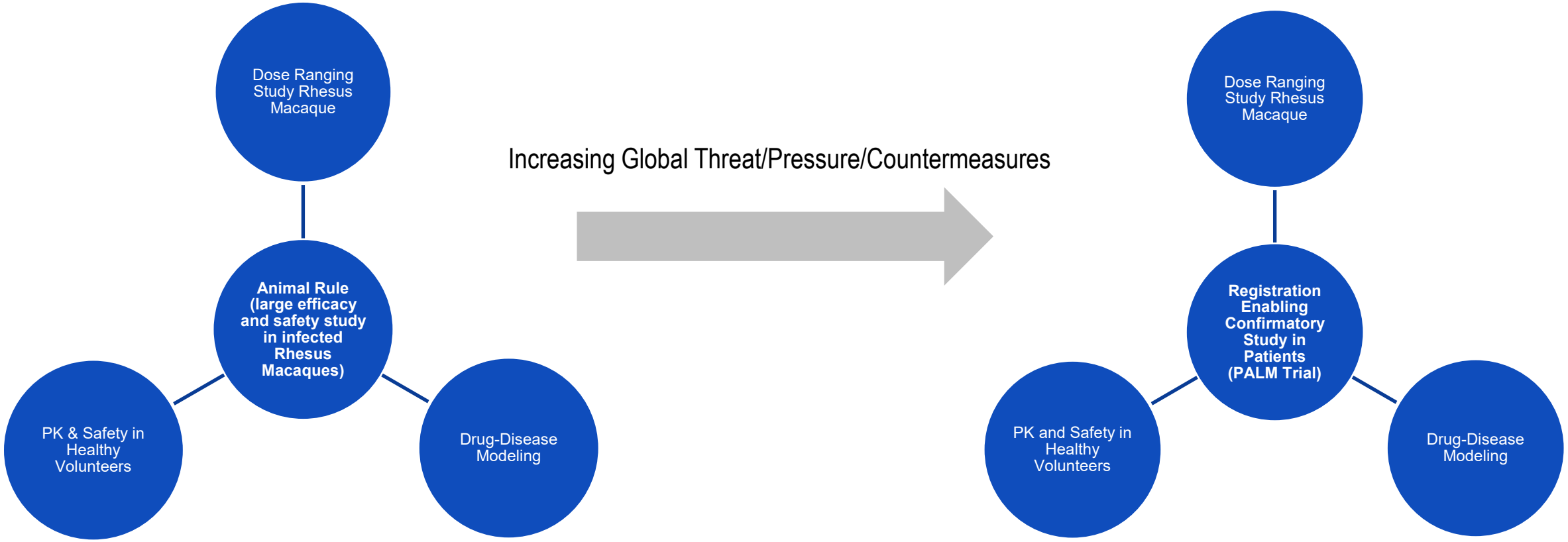
HOW THE REGULATORY & DEVELOPMENT PATHWAY FOR EB3 SHIFTED AS A RESULT OF INCREASING GLOBAL PUBLIC HEALTH CONCERNS OF EBOLA



FUTURE APPLICATIONS OF THE MODULAR TRANSLATIONAL APPROACH

- **Hypothesis testing and as a personalized prediction tool**
  - testing the effect of combination therapies
  - symptom onset timing
  - higher baseline viral load
- **Informing Emergency Use/outbreak Readiness Decisions**
  - Pre-emptive stockpiling
  - Dose adjustments in severe outbreaks
  - Pediatric bridging
- **As a template for mechanistic drug-disease modelling of novel therapies in other highly infectious diseases**
  - Translational pharmacology to rare or deadly pathogens where clinical trials are impossible (e.g. smallpox)
  - Integrating data generated using the animal rule where human data is sparse
  - Optimizing dose selection for registrational trials if the regulatory pathway changes as global threats emerge
- **Variant Specific Dosing**
  - Accounting for resistance
  - simulating survival under mutated viral characteristics without needing large NHP studies

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- Sumathi Sivapalasingam
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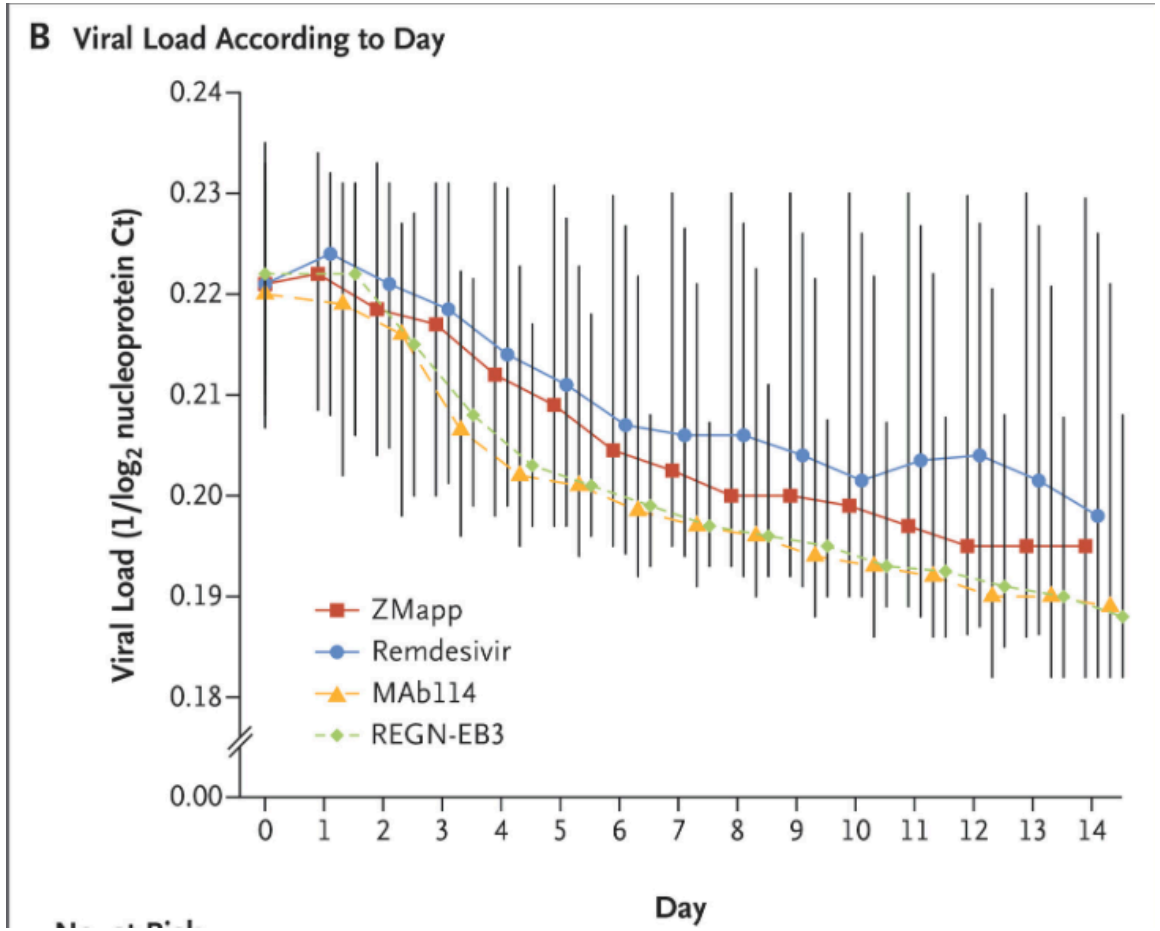
- Nidal Huniti
- Kenneth Turner
- Maria Rosario

BACK- UP- HUMAN VIRAL LOAD PALM TRIAL



Mulangu et al, N Engl J Med 2019;381:2293-2303