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# anti-IL-6 *versus* anti-IL-6R Blocking Antibodies to Treat Acute Ebola Infection in BALB/c Mice with Potential Implications for Treating Patients Presenting with COVID-19

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## ABSTRACT

Cytokine release syndrome (CRS) is known to be a factor in morbidity and mortality associated with acute viral infections including those caused by filoviruses and coronaviruses. IL-6 has been implicated as a cytokine negatively associated with survival after filovirus infection. However, IL-6 has also been shown to be an important mediator of innate immunity, important for the host response to an acute viral infection. Clinical studies are now being conducted by various researchers to evaluate the possible role of IL-6 blockers to improve outcomes in critically ill patients with SARS-CoV-2 infection. Most of these studies involve the use of anti-IL-6R monoclonal antibodies (mAbs). We present data showing that direct neutralization of IL-6 with an anti-IL-6 mAb in a BALB/c Ebolavirus (EBOV) challenge model produced a statistically significant improvement in outcome compared with controls when administered within the first 24 hours of challenge and repeated every 72 hours. A similar effect was seen in mice treated with the same dose of anti-IL-6R mAb when the treatment was delayed 48 hrs post-challenge. These data suggest that direct neutralization of IL-6, early during the course of infection, may provide additional clinical benefits to IL-6 receptor blockade alone during treatment of patients with virus-induced CRS. These results may have implications for selecting and managing IL-6 blockade therapy for patients with COVID-19.

**Keywords: Ebola, COVID-19, SARS-CoV-2, IL-6, IL-6R, CRS, Sarilumab, Tocilizumab, filovirus, coronavirus, anti-IL-6 dosing**

## 1 INTRODUCTION

Under normal circumstances, interleukin-6 (IL-6) is secreted transiently by myeloid cells as part of the innate immune response to injury or infections. However, unregulated synthesis and secretion of IL-6 has contributed to a host of pathological effects such as rheumatoid arthritis. (Swaak et al., 1988) Furthermore,

IL-6 induces differentiation of B cells and promotes CD4+ T cell survival during antigen activation and inhibits TGF-beta differentiation, providing a crucial link between innate and acquired immune responses. (Korn et al., 2008; Dienz and Rincon, 2009) These actions place IL-6 in a central role in mediating and amplifying cytokine release syndrome, commonly associated with Ebola and SARS-CoV-2 infections. (Wauquier et al., 2010; Conti et al., 2020). Patients with COVID-19, the disease caused by infection with SARS-CoV-2, can present with debilitating pneumonia and other complications including acute respiratory distress syndrome (ARDS) (Zhou et al., 2020; Chen et al., 2020; Huang et al., 2020a; Lescure et al., 2020). Elevated IL-6 was found to be significantly correlated with death in COVID-19 patients (Ruan et al., 2020). Originally developed for the treatment of arthritis, anti-IL-6R mAbs have been used to treat CRS as a complication of cancer therapy using adaptive T-cell therapies. (Tanaka et al., 2016; Ascierto et al., 2020; Lee et al., 2014). Warnings admonishing the use of IL-6 blockers in the context of acute infection are present in the package inserts for tocilizumab (Genentech, 2014), sarilumab (Sanofi, 2017) and siltuximab (EUSA, 2015). However, the potential value of using these biologics to treat COVID-19 patients was discussed early during the SARS-CoV-2 outbreak (Mehta et al., 2020a; Liu et al., 2020).

Ebola virus infection is well known to produce CRS, and IL-6 serum levels are known to be inversely correlated with survival in patients post-infection (Wauquier et al., 2010). Recent evidence suggests that patients with clinically severe SARS-CoV-2 infection might also have a CRS syndrome (Huang et al., 2020b). Similarly, the severity of SARS-CoV-1 infection has been shown to be associated with increased serum concentrations of IL-6, leading clinical scientists to propose non-corticosteroid based immunosuppression by using IL-6 blockade as a means to treat hyper inflammation observed in certain patients with SARS-CoV-2 infections (Mehta et al., 2020b; Wong et al., 2004). Indeed, a recent (5/24/2020) search of ClinicalTrials.gov revealed at least 62 clinical trials examining the efficacy and safety of anti-IL-6R monoclonal antibodies (mAbs) and anti-IL-6 mAb for management of patients with COVID-19 (45 studies for tocilizumab (anti-IL-6R mAb), 14 for sarilumab (anti-IL-6R mAb) and 3 for siltuximab (anti-IL-6 mAb)). Early mixed results of CRS treatment with IL-6 blockers (Herper, 2020; ClinicalTrialsGenetech, 2020; ClinicalTrialsEUSA, 2020; Taylor, 2020; Saha et al., 2020), and our own observations of the role of IL-6 in morbidity and mortality associated with Ebola virus infection (Herst et al., 2020), led us to evaluate the clinical effects of treatment with not only antibody directed against the IL-6 receptor (anti-IL-6R mAb), but also with mAb directed to IL-6 itself (anti-IL-6 mAb). We report here on the observed differences between treatments with anti-IL-6R and anti-IL-6 mAbs and comment on how IL-6 blockade may be relevant to the management and therapy for patients with Ebola infection as well as patients infected with SARS-CoV-2.

## 2 METHODS

### 2.1 Virus Strain

For *in-vivo* experiments, a well-characterized mouse-adapted Ebola virus (maEBOV) stock (Bray et al., 1998; Lane et al., 2019), was used for all studies. All work involving infectious maEBOV was performed in a biosafety level (BSL) 4 laboratory, registered with the Centers for Disease Control and the Prevention Select Agent Program for the possession and use of biological select agents.

### 2.2 Animal Studies

Animal studies were conducted at the University of Texas Medical Branch (UTMB), Galveston, TX in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animal research. UTMB is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and has an approved OLAW Assurance. BALB/c mice (Envigo; n = 146) were challenged with 100 plaque forming units (PFU) of mouse-adapted Ebolavirus (maEBOV) via

intraperitoneal (i.p.) injection as described previously (Comer et al., 2019; Hodge et al., 2016). Experimental groups of 10 mice each were administered anti-IL-6 mouse monoclonal antibody (BioXCell, BE0046, Lebanon, NH) or anti-IL-6R mouse monoclonal antibody (BioXCell, BE0047) at a dose of 100 $\mu g$ in sterile saline via intravenous (i.v.) administration via an indwelling central venous catheter, or 400 $\mu g$ via i.p. injection at 24, 48, or 72 hours post-challenge. Antibody dosing was performed once for the i.v. group or continued at 72-hour intervals for the i.p. groups resulting in a total of four doses over the 14-day study period as summarized in Figure 1. Control mice (n=36) were challenge with maEBOV in parallel, but were treated with antibody vehicle alone.

### 2.3 *In-Vivo* Clinical Observations and Scoring

Following maEBOV challenge, mice were examined daily and scored for alterations in clinical appearance and health as previously described (Lane et al., 2019). Briefly, mice were assigned a score of 1 = Healthy; score 2 = Lethargic and/or ruffled fur (triggers a second observation); score 3 = Ruffled fur, lethargic and hunched posture, orbital tightening (triggers a third observation); score 4 = Ruffled fur, lethargic, hunched posture, orbital tightening, reluctance to move when stimulated, paralysis or greater than 20% weight loss (requires immediate euthanasia) and no score = deceased (Table S1 in Supplemental Materials).

### 2.4 Statistical Methods

Descriptive and comparative statistics including arithmetic means, standard errors of the mean (SEM), Survival Kaplan-Meier plots and Log-rank (Mantel-Cox) testing, D'Agostino & Pearson test for normality, Area-Under-The-Curve and $Z$ Statistics were calculated using R with data from GraphPad Prism files. The clinical composite score data used to calculate the AUC measures were normally distributed. The significance of comparisons ($P$ values) of AUC data was calculated using the $Z$ statistic. $P$ values $< .05$ were considered statistically significant.

## 3 RESULTS

Following maEBOV challenge, mice were dosed i.v. with monoclonal antibody at 24, 48 or 72 hours post-challenge with a single dose of anti-IL-6R mAb, or an initial i.p. dose of anti-IL-6 or anti-IL-6R mAb, followed by additional i.p. doses at 72 hour intervals for a total of four doses. Mice were observed for up to 14 days. The survival and average clinical score for mice receiving a single i.v. dose of anti-IL-6R mAb is shown in Figure 1 in Supplemental Materials (top and middle panel).

The survival patterns for treated and untreated groups following maEBOV challenge were statistically different and most untreated mice succumbed to maEBOV infection by day seven. Because neither survival score alone or average clinical score represented the overall possible clinical benefits of mAb treatment, a secondary composite outcome measure was calculated from the quotient of mouse survival and the average clinical score for each day, similar to that previously reported (Kaempf et al., 2019). We then summed these scores across the last 12 days of observation to create an AUC Survival/Clinical Score (Figure 3). The $Z$ statistic and significance level for this metric was calculated for each experimental condition.

The AUC Survival/Clinical Score showed a minor clinical benefit ($P$ <0.01) when mice were given one 100$u$g dose of anti-IL-6R mAb via central venous catheter at 72 hours after maEBOV challenge, relative to vehicle alone, using the experimental design described in Table S2 in Supplementary Materials. Since the maEBOV challenge was administered intraperitoneally and murine peritoneal macrophages represent a significant depot of cells (Cassado et al., 2015) able to produce IL-6 (Vanoni et al., 2017) following TLR activation, we next compared the activities of anti-IL-6 and anti-IL-6R mAb administered intraperitoneally following maEBOV challenge (Figure 2, and 3). We observed significant differences in the AUC Survival/Clinical Score when anti-IL-6R mAb was administered 48 hours post maEBOV challenge

and then repeated three times at 72 hour intervals. The most significant effect on the AUC Survival/Clinical Score was seen when anti-IL-6 mAb was administered beginning at 24 hours post maEBOV challenge, and then repeated three times at 72 hour intervals.

## 4 DISCUSSION

These data suggest that anti-IL-6 antibody therapy may have a clinical advantage over anti-IL-6R mAb particularly when given early during the course of maEBOV infection. It may also be the case that the observed clinical benefit is associated with incomplete blockade of IL-6 early during the course of the infection allows some innate immune protection against the virus. A comparison of the clinical benefits of anti-IL-6 mAb versus anti-IL-6R, or combined early anti-IL-6 mAb and later anti-IL-6R mAb *versus* either mAb alone, would be interesting to evaluate the potential of IL-6 pathway blockade in the context of Ebola and SARS-CoV-2 infection.

Although antibody blood levels were not obtained during the mouse studies described here, we present a pharmacokinetic model based on literature values (Sanofi, 2017; EUSA, 2015; Medesan et al., 1998) shown in Table S5 in Supplemental Materials. Simulated PK curves for each of the three experiments described is shown in Figure 4. Dosing anti-IL-6 mAb at 24 hours after challenge produced a clinical benefit, whereas dosing anti-IL-6R beginning at the same time point did not. The shorter terminal half-life of anti-IL-6 mAb ($T_{1/2}$ = 57$h$) *versus* anti-IL-6R mAb ($T_{1/2}$ = 223$h$) may help explain why giving anti-IL-6 mAb early after infection provided the most observed clinical benefit. As can be seen from the simulated PK profile in Figure 4 (c), repeated dosing every 72 hours, beginning 24 hours after challenge, is predicted to maintain blood levels peaking at about 200 $\mu g/ml$. This is in contrast to blood levels predicted after similar dosing of anti-IL-6R where the blood levels continue to increase over the study period. These differences seen in the simulated PK profiles may have allowed anti-IL-6 mAb to partially block IL-6, allowing innate immunity to develop, while still providing sufficient blockade to reduce the deleterious clinical effects of IL-6 as the study progressed. In addition, it may be that the stoichiometry of anti-IL-6 blockade *versus* anti-IL-6R may favor achieving partial blockade early during the evolution of CRS given that the amount of IL-6 present may exceed the number of IL-6 receptors. It is also possible that IL-6 may act on other sites not blocked by anti-IL-6R mAb, and that this may yield a potential advantage of using anti-IL-6 mAb to treat CRS brought about by a viral infection.

It may be possible to develop a controlled release formulation of anti-IL-6 mAb to obtain a clinically beneficial effect from the administration of anti-IL-6 mAb, anti-IL-6R mAb, or a combination of both, after a single injection early during the course of SARS-CoV-2 infection. For example, Figure 4(d) shows various predicted controlled release PK profiles of anti-IL-6 mAb that could be achieved by using delivery systems producing different first order rates of delivery from an injection depot of 20mg/Kg. Correlation of these release profiles with the AUC Survival/Clinical score described here in pre-clinical models could lead to the development of a single dose treatment mitigating the effects of CRS on the host. A single dose, controlled release injectable formulation of anti-IL-6 mAb could allow treatment early during the diagnosis of COVID-19, potentially allowing patients to begin receiving therapy early during the evolution of CRS, even before hospitalization.

## 5 CONCLUDING REMARKS

Although the previous reports of use of IL-6 blockers to treat CRS have shown mixed results, recent clinical data for anti-IL-6 and anti-IL-6R mAbs have shown early promise in clinical trials (Gritti et al., 2020; Xu et al., 2020). Pre-clinical studies and various ongoing clinical trials evaluating the potential benefit of IL-6

blockers for the treatment of patients with acute SARS-CoV-2 infection may provide clinical correlation with the results presented here.

## CONFLICT OF INTEREST STATEMENT

Reid Rubsamen, Scott Burkholz, Richard Carback, Tom Hodge, Lu Wang, and Charles Herst are employees of Flow Pharma, Inc. compensated in cash and stock, and are named inventors on various issued and pending patents assigned to Flow Pharma. Some of these patents pending are directly related to the study presented here. Paul Harris is a member of Flow Pharma's Scientific Advisory Board. Christopher Massey, and Trevor Brasel have nothing to declare.

## AUTHOR CONTRIBUTIONS

All co-authors participated in study design, data analysis and drafting of the manuscript. Christopher Massey and Trevor Brasel performed the study under BSL-4 conditions and generated the data presented here.

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**Figure 1.** Dosing Schedule for IL-6 and IL-6R mAbs used in this study.

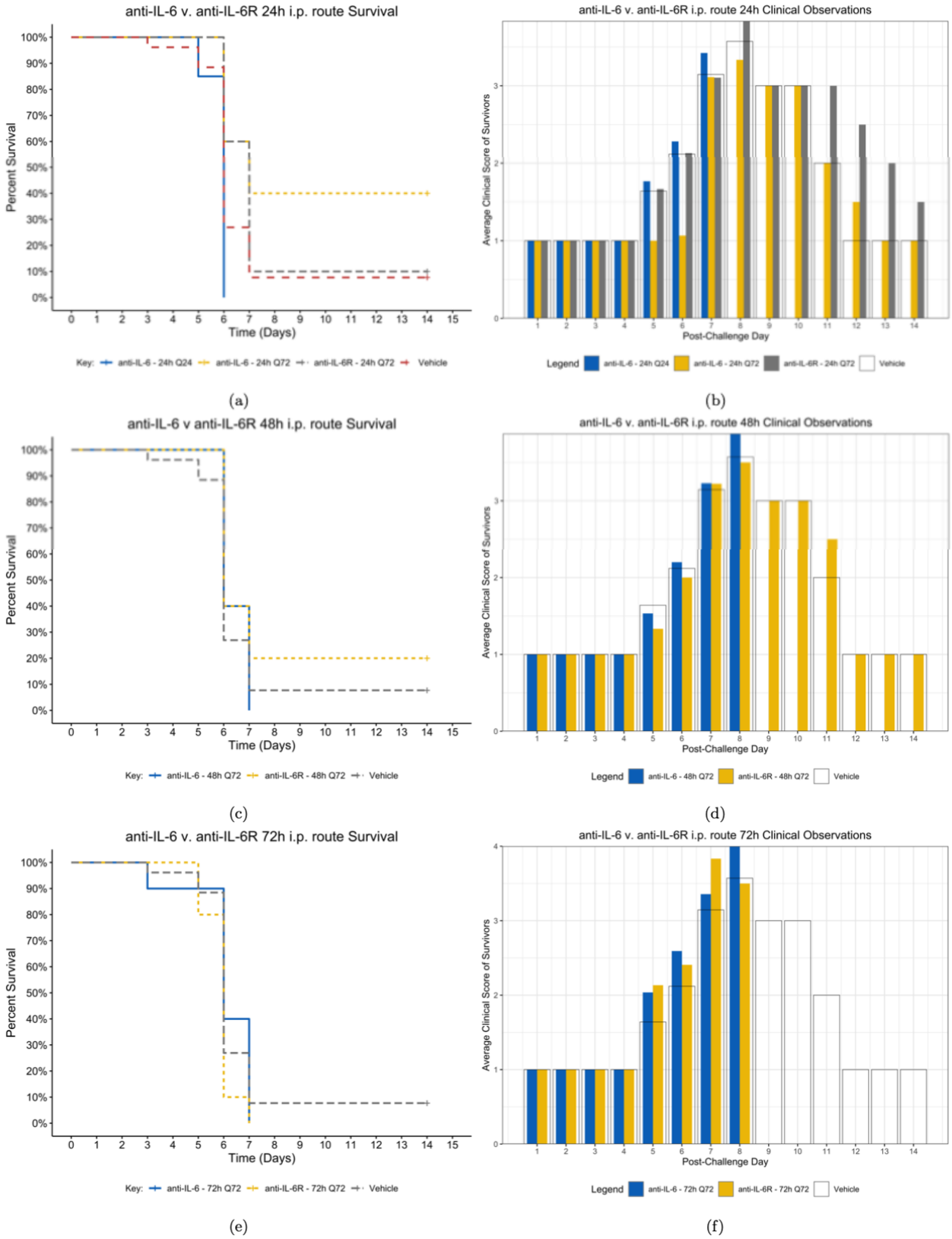


**Figure 2.** Kaplan-Meier Survival Plots and Average clinical scores for a single or multiple i.p. doses of anti-IL-6 or anti-IL-6R administered at 24, 48 or 72 hours following maEBOV challenge followed by repeat dosing every 72 hours for a total of four doses. The survival curves were significantly different by Log-rank (Mantel-Cox) testing ($P$¡0.05). SEM were $< 10\%$ of the mean.

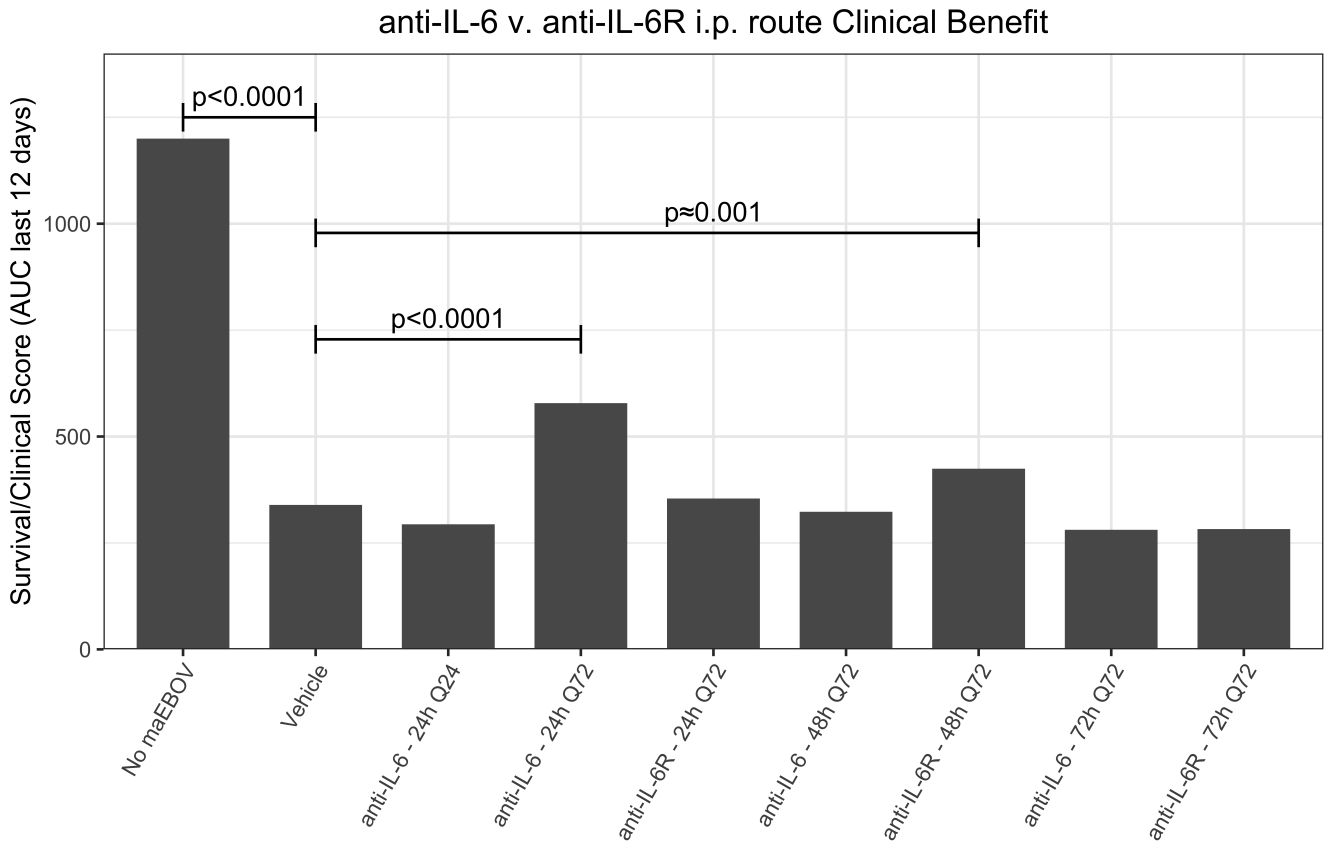


**Figure 3.** A clinical benefit metric was calculated as an area under curve for survival/clinical scores for 120 mice receiving a single or multiple i.p. doses of anti-IL-6 or anti-IL-6R mAb following maEBOV challenge 121 on day 0. The given p values are determined from the Z statistic calculated for each experimental condition.

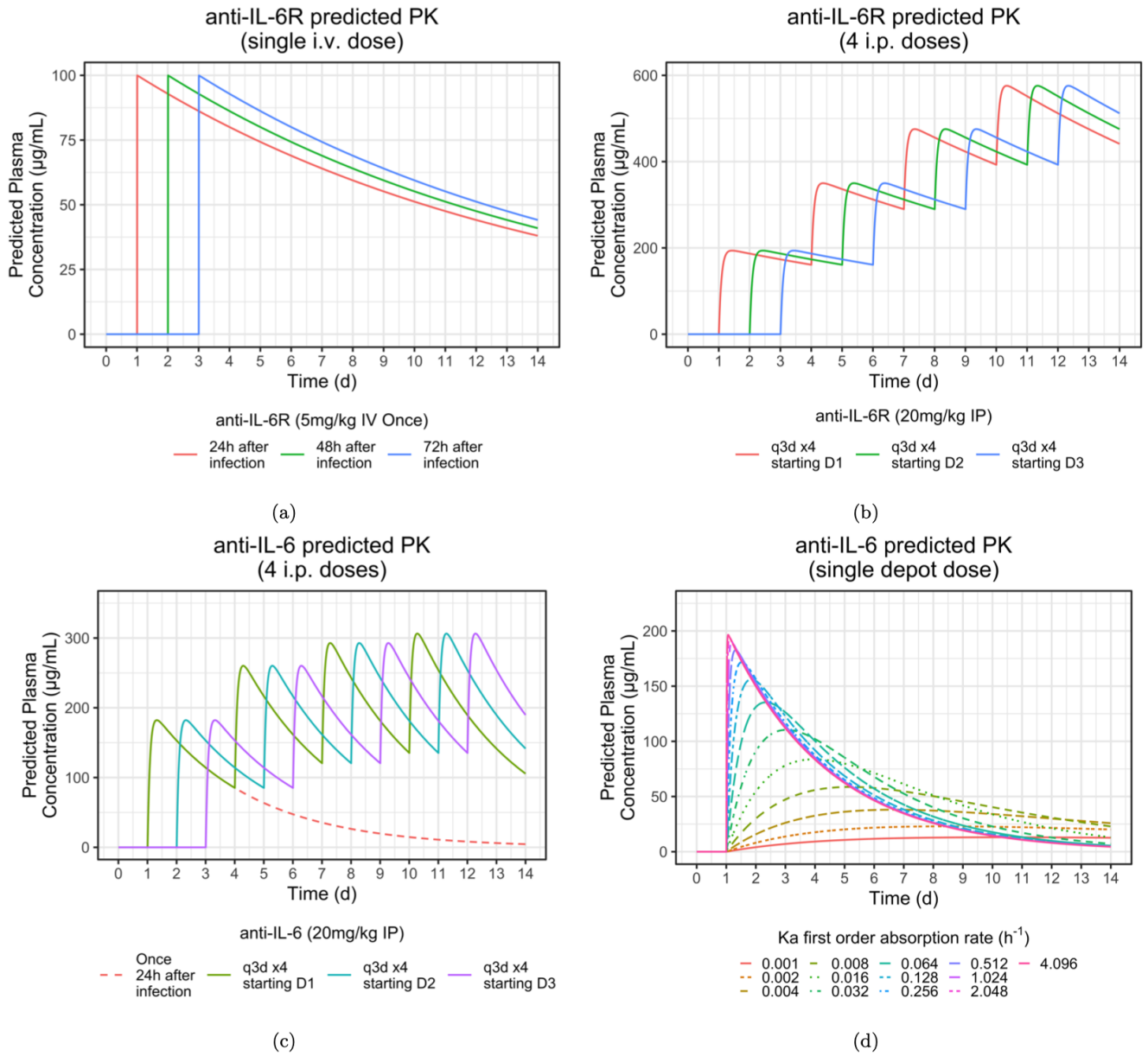


**Figure 4.** Simulated PK profiles for i.v. and i.p. routes of administration based on literature PK parameters shown in Table S5 in Supplemental Materials were determined. Panel (a) models the i.v. delivery experiment. Panels (b) and (c) model i.p. delivery experiments one and two. For each of these simulations, mice were dosed a total of four times at 72 hour intervals, beginning 24 hours after challenge. Panel (d) models release profiles for simulated controlled release scenarios with different absorption rates as indicated by the listed $K_a$ parameters after a single depot injection of 20mg/Kg.