

Anti-IL-6 versus Anti-IL-6R Blocking Antibodies to Treat Acute Ebola Infection in BALB/c Mice: Potential Implications for Treating Cytokine Release Syndrome

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2 ABSTRACT

3 Cytokine release syndrome (CRS) is known to be a factor in morbidity and mortality associated
4 with acute viral infections including those caused by filoviruses and coronaviruses. IL-6 has
5 been implicated as a cytokine negatively associated with survival after filovirus and coronavirus
6 infection. However, IL-6 has also been shown to be an important mediator of innate immunity
7 and important for the host response to an acute viral infection. Clinical studies are now being
8 conducted by various researchers to evaluate the possible role of IL-6 blockers to improve
9 outcomes in critically ill patients with CRS. Most of these studies involve the use of anti-IL-6R
10 monoclonal antibodies (α -IL-6R mAbs). We present data showing that direct neutralization of
11 IL-6 with an α -IL-6 mAb in a BALB/c Ebolavirus (EBOV) challenge model produced a statistically
12 significant improvement in outcome compared with controls when administered within the first
13 24 hours of challenge and repeated every 72 hours. A similar effect was seen in mice treated
14 with the same dose of α -IL-6R mAb when the treatment was delayed 48 hrs post-challenge.
15 These data suggest that direct neutralization of IL-6, early during the course of infection, may
16 provide additional clinical benefits to IL-6 receptor blockade alone during treatment of patients
17 with virus-induced CRS.

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

1 INTRODUCTION

19 Under normal circumstances, interleukin-6 (IL-6) is secreted transiently by myeloid cells as part of the
20 innate immune response to injury or infections. However, unregulated synthesis and secretion of IL-6 has

21 contributed to a host of pathological effects such as rheumatoid arthritis. (Swaak et al., 1988) Furthermore,
22 IL-6 induces differentiation of B cells and promotes CD4+ T cell survival during antigen activation and
23 inhibits TGF-beta differentiation, providing a crucial link between innate and acquired immune responses
24 (Korn et al., 2008; Dienz and Rincon, 2009). These actions place IL-6 in a central role in mediating
25 and amplifying cytokine release syndrome (CRS), commonly associated with Ebola virus disease (EVD)
26 infections. (Wauquier et al., 2010). CRS is known to be a factor in morbidity and mortality associated with
27 acute viral infections including those caused by filoviruses and coronaviruses. For example, non-survivors
28 of the West African EBOV epidemics exhibited significantly elevated levels of the overall inflammatory
29 response cytokines and monokines compared to survivors (Ruibal et al., 2016). It is thought that prolonged
30 exposure to elevated inflammatory cytokine levels is toxic to T cells and results in their apoptotic and necrotic
31 cell death (Younan et al., 2018). Both lymphopenia and elevated serum IL-6 levels are found in Ebola
32 virus infection and are known to be inversely correlated with survival in patients post-infection (Wauquier
33 et al., 2010) and in mouse models of Ebola infection (Herst et al., 2020). However, IL-6 has also been
34 shown to be an important mediator of innate immunity and important for the host recovery from acute viral
35 infection (Yang et al., 2017). Elevated IL-6 levels are also observed in SARS-CoV-2 infections, severe
36 influenza, rhinovirus, RSV infection, as well as in similar respiratory infections (Conti et al., 2020;
37 Hayden et al., 1998; Tang et al., 2016; Kerrin et al., 2017). Originally developed for the treatment of
38 arthritis, α -IL-6R mAbs have been used to treat CRS as a complication of cancer therapy using adaptive
39 T-cell therapies. (Tanaka et al., 2016; Ascierto et al., 2020; Lee et al., 2014). Warnings admonishing the
40 use of IL-6 blockers in the context of acute infection are present in the package inserts for tocilizumab
41 (Genentech, 2014), sarilumab (Sanofi, 2017) and siltuximab (EUSA, 2015). Early mixed results of CRS
42 treatment with IL-6 blockers (Herper, 2020; ClinicalTrialsGenetech, 2020; ClinicalTrialsEUSA, 2020;
43 Taylor, 2020; Saha et al., 2020), and our own observations of the role of IL-6 in morbidity and mortality
44 associated with Ebola virus infection (Herst et al., 2020), led us to evaluate the clinical effects of treatment
45 with not only antibody directed against the IL-6 receptor, but also with mAb directed to IL-6 itself. We
46 report here on the observed differences between treatments with α -IL-6R mAbs and α -IL-6 mAbs in a
47 mouse model of EBOV infection and comment on how IL-6 blockade may be relevant to the management
48 and therapy for patients with Ebola infection as well as patients infected with SARS-CoV-2.

2 METHODS

49 2.1 Virus Strain

50 For *in-vivo* experiments, a well-characterized mouse-adapted Ebola virus (maEBOV) stock (Bray et al.,
51 1998; Lane et al., 2019) (Ebola virus M. musculus/COD/1976/Mayinga-CDC-808012), derived from the
52 1976 Zaire ebolavirus isolate Yambuku-Mayinga (Genebank accession NC002549), was used for all studies.
53 All work involving infectious maEBOV was performed in a biosafety level (BSL) 4 laboratory, registered
54 with the Centers for Disease Control and the Prevention Select Agent Program for the possession and use
55 of biological select agents.

56

57 2.2 Animal Studies

58 Animal studies were conducted at the University of Texas Medical Branch (UTMB), Galveston, TX
59 in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animal
60 research. UTMB is fully accredited by the Association for the Assessment and Accreditation of Laboratory
61 Animal Care International and has an approved OLAW Assurance. BALB/c mice (Envigo; n = 146) were
62 challenged with 100 plaque forming units (PFU) of maEBOV via intraperitoneal (i.p.) injection as described
63 previously (Comer et al., 2019; Hodge et al., 2016). Experimental groups of 10 mice each were administered

64 rat anti-mouse-IL-6 IgG1 monoclonal antibody (BioXCell, BE0046, Lebanon, NH, RRID AB1107709) or
65 rat anti-mouse-IL-6R IgG2 monoclonal antibody (BioXCell, BE0047, RRID AB1107588) at a dose of 100
66 μ g in sterile saline via intravenous (i.v.) administration via an indwelling central venous catheter, or 400 μ g
67 via i.p. injection at 24, 48, or 72 hours post-challenge. Antibody dosing was based on amounts previously
68 reported to neutralize IL-6 and IL-6R in mice (Liang et al., 2015; DL et al., 2014). Antibody dosing was
69 performed once for the i.v. group or continued at 72-hour intervals for the i.p. groups resulting in a total
70 of four doses over the 14-day study period as summarized in Figure 1 and Tables S2-S5 (Supplemental
71 Materials). Control mice (n=36) were challenge with maEBOV in parallel, but were treated with antibody
72 vehicle alone. Serum IL-6 measurements were performed in control rodents at necropsy as previously
73 described (Herst et al., 2020).

74

75 **2.3 In-Vivo Clinical Observations and Scoring**

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

82

83 **2.4 Statistical Methods**

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

3 RESULTS

90 Following maEBOV challenge, mice were dosed i.v. at 24, 48 or 72 hours post-challenge with a single
91 dose of α -IL-6R mAb, a single i.p. dose of α -IL-6R mAb 24 hours after maEBOV challenge, or an initial
92 i.p. dose of α -IL-6 or α -IL-6R mAb, followed by additional i.p. doses at 72 hour intervals for a total of
93 four doses. Mice were observed for up to 14 days as summarized in Figure 1. The average serum IL-6
94 concentration at necropsy for mice (n=5) challenged with maEBOV was 1092 ± 505 pg/ml, a concentration
95 similar to that reported in a previous publication for mice challenged with 10 PFU of maEBOV (Chan et al.,
96 2019). In mice not challenged with maEBOV the average serum IL-6 was 31 ± 11 pg/ml. The survival and
97 average clinical score for mice receiving a single i.v. dose of α -IL-6R mAb is shown in Figure S1 (Panel
98 A and Panel B, Supplemental Materials) . Little to no effects on survival or clinical score were observed
99 following maEBOV challenge and a single i.v. dose of α -IL-6R mAb.

100 The survival patterns for i.v. mAb treated and untreated groups following maEBOV challenge were
101 statistically different and most untreated mice succumbed to maEBOV infection by day seven(Figure S1 ,
102 Supplementary Materials). Because neither survival score alone or average clinical score represented the
103 overall possible clinical benefits of mAb treatment, a secondary composite outcome measure was calculated
104 from the quotient of mouse survival and the average clinical score for each day, similar to that previously
105 reported (Kaempf et al., 2019). We then summed these scores across the last 12 days of observation to

106 create an AUC Survival/Clinical Score (see Figure S1, Panel C, Supplemental Materials). The Z statistic
107 and significance level for this metric was calculated for each experimental condition. We found a minor
108 clinical benefit ($P < 0.01$) when mice were given one 100 μ g dose of α -IL-6R mAb via central venous
109 catheter at 72 hours after maEBOV challenge, relative to vehicle alone, using the experimental design
110 described in Table S2 (Supplementary Materials).

111 Since the maEBOV challenge was administered intraperitoneally and murine peritoneal macrophages
112 represent a significant depot of cells (Cassado et al., 2015) able to produce IL-6 (Vanoni et al., 2017)
113 following toll-like receptor activation, we next compared the activities of α -IL-6 and α -IL-6R mAbs
114 administered intraperitoneally following maEBOV challenge (Figures 2, 3, 4, and 5). We observed
115 significant differences in the AUC Survival/Clinical Score when α -IL-6R mAb was administered 48 hours
116 post maEBOV challenge and then repeated three times at 72 hour intervals. The most significant beneficial
117 effect on the AUC Survival/Clinical Score (Figure 5) was seen when α -IL-6 mAb was administered
118 beginning at 24 hours post maEBOV challenge, and then repeated three times at 72 hour intervals.

4 DISCUSSION

119 While EVD is classified as a viral haemorrhagic fever, there are many similarities between EVD and
120 COVID-19, the disease caused by infection with SARS-CoV-2 that can present as an acute respiratory
121 distress syndrome (ARDS) (Zhou et al., 2020; Chen et al., 2020; Huang et al., 2020a; Lescure et al., 2020).
122 Like EVD, elevated IL-6 was found to be significantly correlated with death in COVID-19 patients (Ruan
123 et al., 2020), suggesting that patients with clinically severe SARS-CoV-2 infection might also have a CRS
124 syndrome (Huang et al., 2020b). Both EVD and COVID-19 (Younan et al., 2019; Tan et al., 2020) are
125 associated with lymphopenia. Since the severity of SARS-CoV-1 infection has been shown to be associated
126 with increased serum concentrations of IL-6, clinical scientists have proposed non-corticosteroid based
127 immunosuppression by using IL-6 blockade as a means to treat hyper inflammation observed in certain
128 patients with SARS-CoV-2 infections (Mehta et al., 2020a; Wong et al., 2004). The potential value of using
129 IL-6 blockade to treat COVID-19 patients was discussed early during the 2020 SARS-CoV-2 outbreak
130 (Mehta et al., 2020b; Liu et al., 2020). Indeed, a recent (5/24/2020) search of ClinicalTrials.gov revealed at
131 least 62 clinical trials examining the efficacy and safety of α -IL-6R mAbs and α -IL-6 mAbs for management
132 of patients with COVID-19; 45 studies for tocilizumab (α -IL-6R mAbs), 14 for sarilumab (α -IL-6R mAbs)
133 and 3 for siltuximab (α -IL-6 mAbs). Most of the studies involve the use of α -IL-6R mAbs and have shown
134 promising results (summarized in Tables 1 and 2), but there is clear need for improvement.

135 Using a mouse model of Ebola infection, we found clinical benefit when mice were administered multiple
136 i.p. doses of α -IL-6R mAb 48 hours after maEBOV challenge. At both earlier (24h) and later (72h) time
137 points of initiation of administration of α -IL-6R mAb, we observed little to no effects on the clinical benefit
138 score. Similarly, we found clinical benefit when α -IL-6 mAb was administered beginning at 24 hours post
139 maEBOV challenge, and then repeated three times at 72 hour intervals, but no benefit was observed if
140 α -IL-6 mAb was initiated at 48 or 72 hours post challenge. These data suggest that α -IL-6 mAb therapy
141 may also have clinical benefits similar to α -IL-6R mAb particularly when given early during the course of
142 maEBOV infection.

143 Previous experiments in the murine EBOV system (Herst et al., 2020) suggest that some degree of
144 activation of innate immunity and IL 6 release benefits survival post maEBOV challenge. It may be the
145 case that the observed clinical benefits of α -IL-6 mAbs are associated with incomplete blockade of the
146 IL-6 response particularly later than 24 post challenge. Overall our data suggest that human clinical trials
147 evaluating the benefits of α -IL-6 mAbs versus α -IL-6R mAbs versus combined early α -IL-6 mAb and

148 later α -IL-6R mAb is warranted to evaluate the potential of IL-6 pathway blockade in the during Ebola or
149 SARS-CoV-2 infection.

150 Although antibody blood levels were not obtained during the mouse studies described here, we present a
151 pharmacokinetic model based on literature values (Sanofi, 2017; EUSA, 2015; Medesan et al., 1998) shown
152 in Table S5 in Supplemental Materials. Simulated PK curves for each of the three experiments described is
153 shown in Figure 6. Dosing α -IL-6 mAb at 24 hours after challenge produced a clinical benefit, whereas
154 dosing α -IL-6R beginning at the same time point did not. The shorter terminal half-life of α -IL-6 mAb ($T_{1/2}$
155 = 57h) *versus* α -IL-6R mAb ($T_{1/2}$ = 223h), possibly due to isotype specific differences in glycosylation
156 (Cobb, 2019) may help explain why giving α -IL-6 mAb early after infection provided the most observed
157 clinical benefit. As can be seen from the simulated PK profile in Figure 6 (c), repeated dosing every 72
158 hours, beginning 24 hours after challenge, is predicted to maintain blood levels peaking at about 200
159 μ g/ml. This is in contrast to blood levels predicted after similar dosing of α -IL-6R where the blood levels
160 continue to increase over the study period. These differences seen in the simulated PK profiles may have
161 allowed α -IL-6 mAb to partially block IL-6, allowing innate immunity to develop, while still providing
162 sufficient blockade to reduce the deleterious clinical effects of IL-6 as the study progressed. In addition, it
163 may be that the stoichiometry of α -IL-6 blockade *versus* α -IL-6R may favor achieving partial blockade
164 early during the evolution of CRS given that the amount of IL-6 present may exceed the number of IL-6
165 receptors. It is also possible that IL-6 may act on other sites not blocked by α -IL-6R mAb, and that this
166 may yield a potential advantage of using α -IL-6 mAb to treat CRS brought about by a viral infection.

167 It may be possible to develop a controlled release formulation of α -IL-6 mAb to obtain a clinically
168 beneficial effect from the administration of α -IL-6 mAb, α -IL-6R mAb, or a combination of both, after a
169 single injection early during the course of SARS-CoV-2 infection. For example, Figure 6, bottom-right
170 panel, shows various predicted controlled release PK profiles of α -IL-6 mAb that could be achieved by
171 using delivery systems producing different first order rates of delivery from an injection depot of 20mg/Kg.
172 Correlation of these release profiles with the AUC Survival/Clinical score described here in pre-clinical
173 models could lead to the development of a single dose treatment mitigating the effects of CRS on the host.

5 CONCLUDING REMARKS

174 Although the previous reports of use of IL-6 blockers to treat CRS have shown mixed results, recent
175 clinical data for α -IL-6 and α -IL-6R mAbs have shown early promise in human trials for treatment of
176 severe influenza and corona virus infections (Gritti et al., 2020; Xu et al., 2020). Pre-clinical studies and
177 various ongoing clinical trials evaluating the potential benefit of IL-6 blockers, for example, early α -IL-6
178 mAb and later α -IL-6R mAb, for the treatment of patients with CRS may provide clinical correlation with
179 the results presented here.

CONFLICT OF INTEREST STATEMENT

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

AUTHOR CONTRIBUTIONS

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

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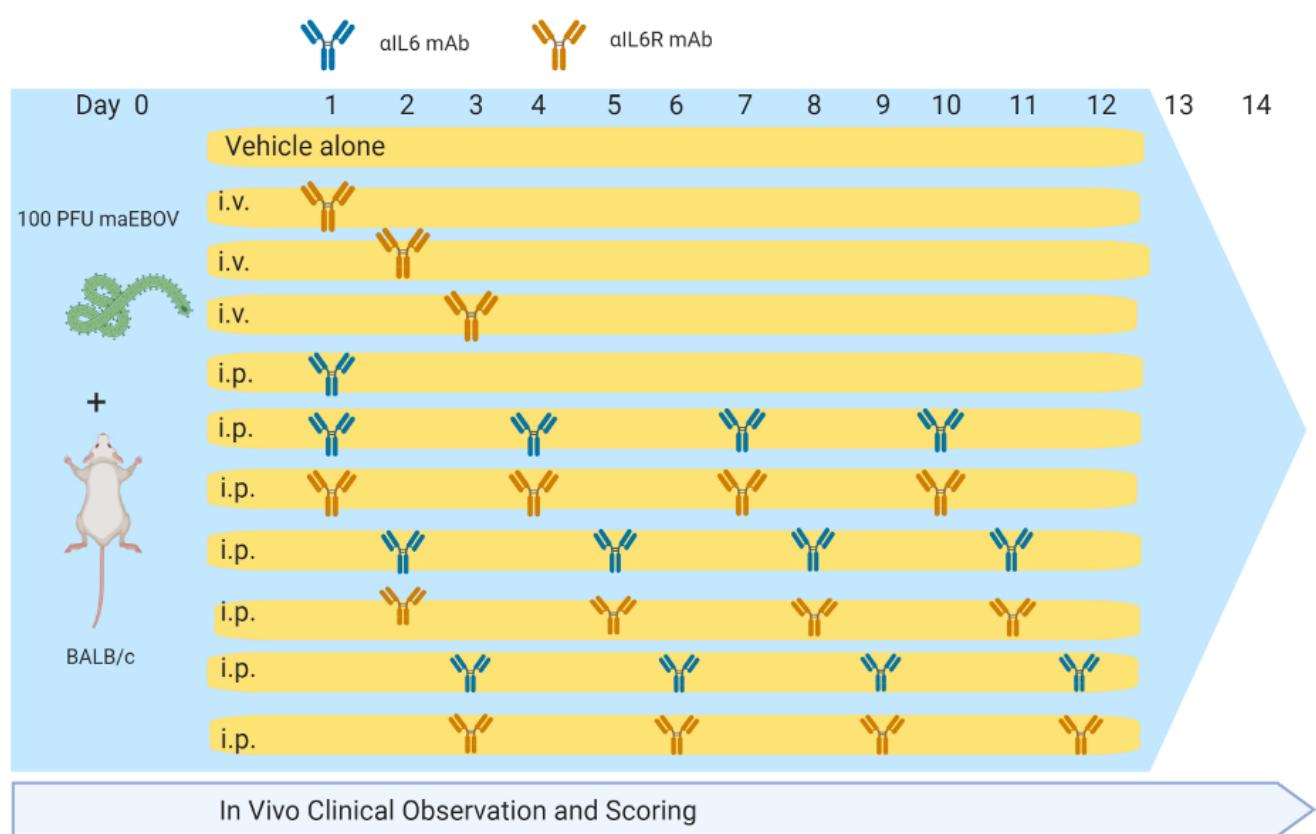


Figure 1. Dosing Schedule for α -IL-6 and α -IL-6R mAbs used in this study.

Patient Population	Design, Number of Patients, and Primary Outcomes	Treatment/Dose	Conclusions and Reference
RT-PCR confirmed Sars Cov-2 pneumonia, SpO ₂ <93% in room air or mechanical ventilation	PROSPECTIVE TWO ARMS: Standard of Care (n=365) and Standard of Care plus Tocilizumab (n=179) OUTCOME: Survival	Tocilizumab (α -IL-6R) i.v. 8mg/Kg in two infusions 12h apart not exceeding 800mg total	Significantly improved survival associated with use of Tocilizumab($p<0.001$) Guaraldi et al. (2020)
RT-PCR confirmed Sars Cov-2 pneumonia, SpO ₂ <93% in room air or mechanical ventilation	PROSPECTIVE SINGLE ARM: Severe Disease versus Non-Severe Disease (n=239) OUTCOME: Clinical parameters and historical survival	Tocilizumab (α -IL-6R) i.v. 8mg/Kg not exceeding 800mg total	Tocilizumab-treated patients with severe disease had survival similar to that of Tocilizumab-treated patients with nonsevere disease. Price et al. (2020)
RT-PCR confirmed Sars Cov-2 pneumonia, SpO ₂ <93% in room air, ICU admission with or without mechanical ventilation	PROSPECTIVE TWO ARMS: Standard of Care (n=420) and Standard of Care plus Tocilizumab (n=210) OUTCOME: Survival	Tocilizumab (α -IL-6R) i.v. one or two doses of 400mg	Patients receiving Tocilizumab had significantly decreased hospital-related mortality ($p<0.004$) Biran et al. (2020)
Clinical Diagnosis of COVID-19	RETROSPECTIVE SINGLE ARM: Pre- and Post-Tocilizumab outcome (n=15) OUTCOME: Clinical parameter: CRP level	Tocilizumab (α -IL-6R) i.v. 80-600mg once or multi 80-160mg doses	Reduced C-Ractive protein levels relative to pretreatment levels Luo et al. (2020)
RT-PCR confirmed Sars Cov-2 pneumonia, SpO ₂ <90% in room air	PROSPECTIVE SINGLE ARM: Pre- and Post-Tocilizumab (n=100) OUTCOME: Clinical parameters: BCRSS respiratory score	Tocilizumab (α -IL-6R) i.v. 8mg/Kg in two doses 12h apart. Discretionary third dose.	Improvement of clinical symptoms and reduced BCRSS scores associated with treatment with Tocilizumab. Toniati et al. (2020)
RT-PCR and X-ray confirmed Sars Cov-2 pneumonia, SpO ₂ <90% in room air	RETROSPECTIVE CASE-CONTROL STUDY: Standard of Care (n=25) and Standard of Care plus Tocilizumab (n=20) OUTCOME: Survival	Tocilizumab (α -IL-6R) i.v. once or twice	Significantly Improved survival associated with administration of Tocilizumab ($p<0.002$). Klopfenstein et al. (2020)
RT-PCR confirmed Sars Cov-2 pneumonia, SpO ₂ <93% in room air requiring mechanical ventilation	PROSPECTIVE TWO ARMS: Standard of Care (n=76) and Standard of Care plus Tocilizumab (n=78) OUTCOME: Survival	Tocilizumab or Sarilumab (α -IL-6R) i.v. 8mg/Kg not exceeding 800mg total	Improved survival associated with administration of Tocilizumab deduced from 45% reduction in hazard of death [hazard ratio 0.55 (95% CI 0.33, 0.90)]. Somers et al. (2020)

Table 1. Summary of recent literature on use of α IL-6R mAb for treatment of SARS-CoV-2 infection. (1 of 2)

Patient Population	Design, Number of Patients, and Primary Outcomes	Treatment/Dose	Conclusions and Reference
RT-PCR confirmed Sars Cov-2 pneumonia, SpO ₂ <92% in room air	PROSPECTIVE SINGLE ARM: Pre- and Post-Tocilizumab (n=63) OUTCOME: Clinical parameters (CRP levels and ratio PaO ₂ /FiO ₂)	Tocilizumab (α -IL-6R) i.v. 8mg/Kg not exceeding 800mg total once or twice	Improvement in clinical parameters. Sciascia et al. (2020)
RT-PCR and X-Ray confirmed Sars Cov-2 pneumonia, SpO ₂ <93%	PROSPECTIVE TWO ARMS: Standard of Care (n=28) and Standard of Care plus Tocilizumab (n=28) OUTCOME: Survival	Tocilizumab (α -IL-6R) i.v. 400mg total	No significant improvement in clinical parameters, but faster recovery in subset with less severe disease. Della-Torre et al. (2020)
RT-PCR confirmed Sars Cov-2 pneumonia, SpO ₂ <93% in room air or mechanical ventilation	PROSPECTIVE SINGLE ARM: Pre- and Post-Tocilizumab (n=15) OUTCOME: Clinical parameters	Sarilumab (α -IL-6R) s.c. 400mg one or two doses	Rapid improvement in clinical and biochemical outcomes responders (%66), but (33%) were non-responders. Montesarchio et al. (2020)
RT-PCR confirmed Sars Cov-2 pneumonia. SpO ₂ <92%	PROSPECTIVE SINGLE ARM with two subgroups (A (n=149): requiring FiO ₂ <45% and B (n=106): requiring FiO ₂ >45%) OUTCOME: Survival	Tocilizumab (α -IL-6R) i.v. 400mg or Sarilumab (α -IL-6R) i.v. 400mg given once or twice	Improved survival in patients with severe disease (subgroup A) as compared to the subgroup B suggests that anti-IL-6 R intervention should occur prior to the onset of critical illness for maximum benefit. Sinha et al. (2020)

Table 2. Summary of recent literature on use of α IL-6R mAb for treatment of SARS-CoV-2 infection. (2 of 2)

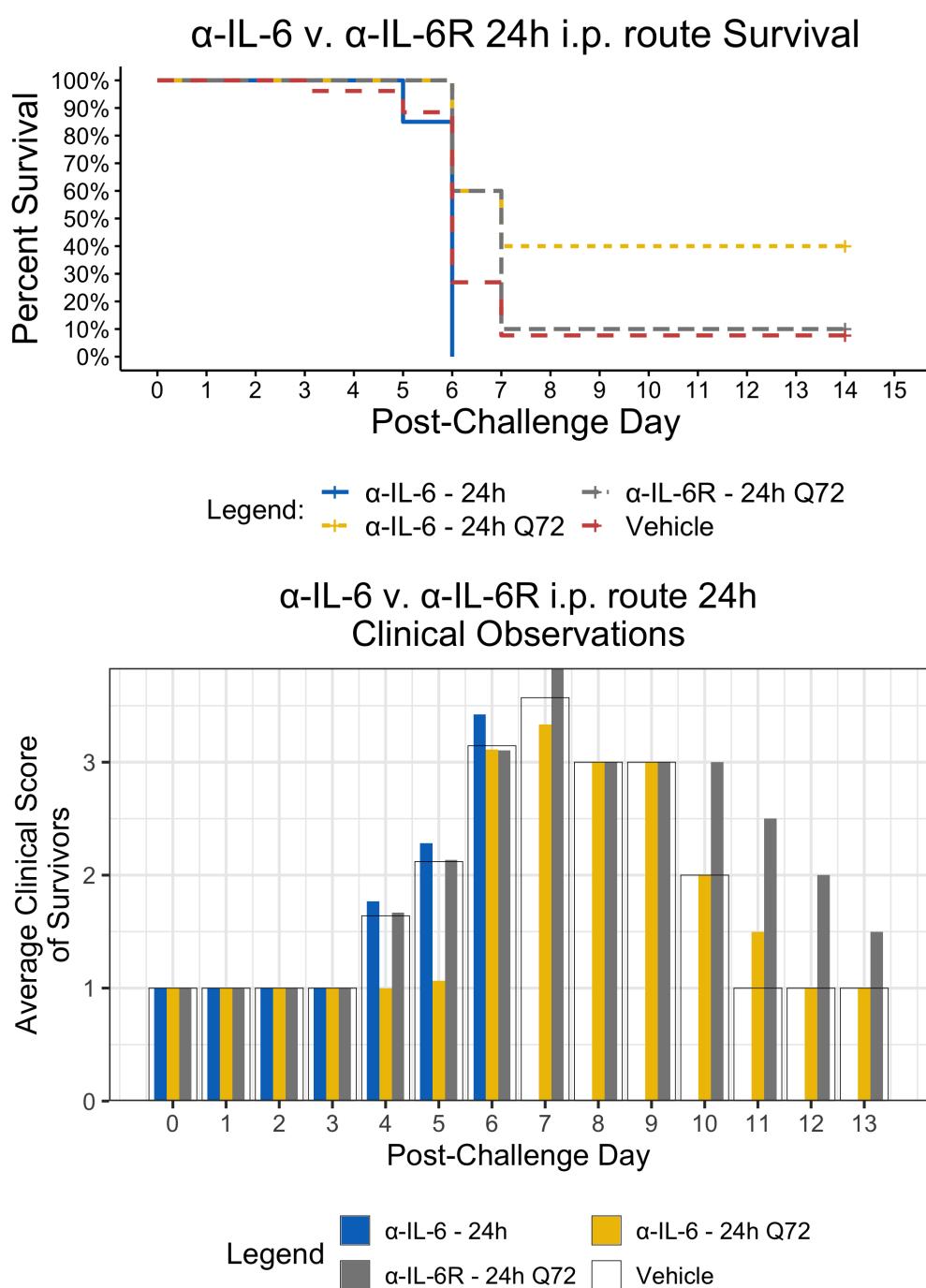


Figure 2. Kaplan-Meier Survival Plots and Average clinical scores for a single or multiple i.p. doses of α -IL-6 or α -IL-6R administered 24 hours after maEBOV challenge and followed by repeat dosing every 72 hours for a total of four doses. The survival curves were significantly different by Log-rank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

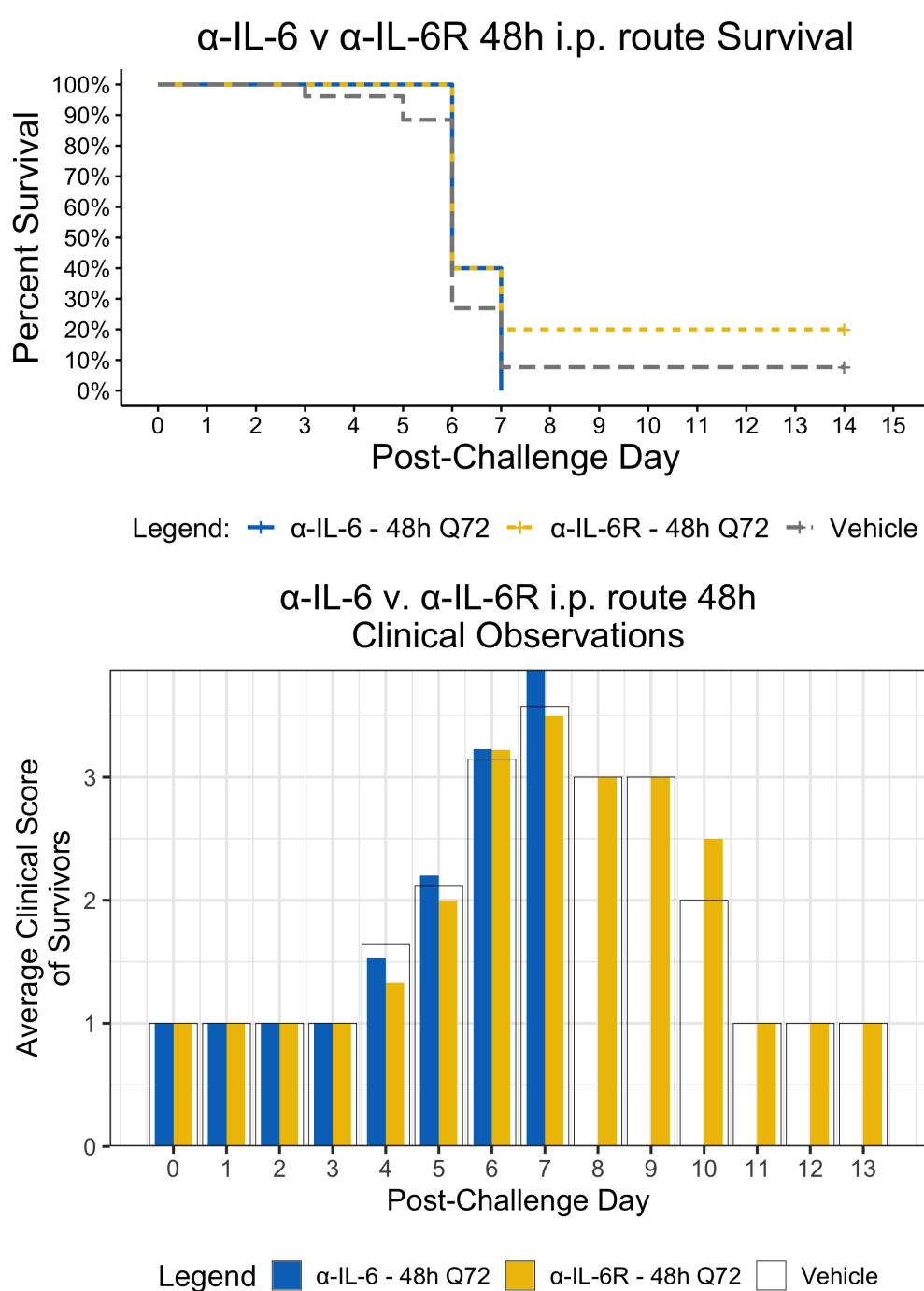


Figure 3. Kaplan-Meier Survival Plots and Average clinical scores for multiple i.p. doses of α -IL-6 or α -IL-6R administered 48 hours after maEBOV challenge and followed by repeat dosing every 72 hours for a total of four doses. The survival curves were significantly different by Log-rank (Mantel-Cox) testing ($P < 0.05$). SEM of the average clinical scores were < 10% of the mean.

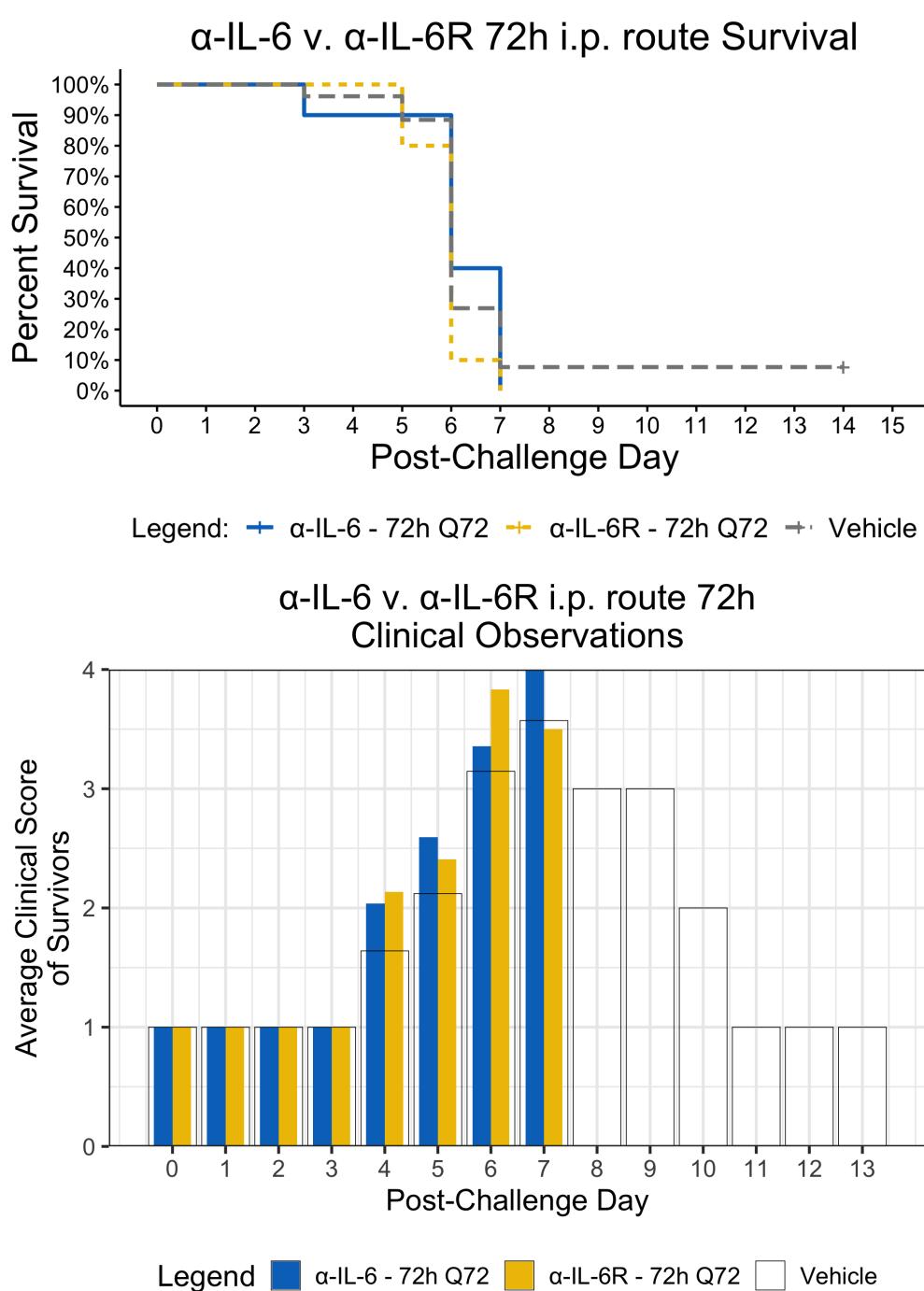


Figure 4. Kaplan-Meier Survival Plots and Average clinical scores for multiple i.p. doses of α -IL-6 or α -IL-6R administered 72 hours after maEBOV challenge and followed by repeat dosing every 72 hours for a total of four doses. The survival curves were significantly different by Log-rank (Mantel-Cox) testing ($P < 0.05$). SEM of the average clinical scores were < 10% of the mean.

α -IL-6 v. α -IL-6R i.p. route Clinical Benefit

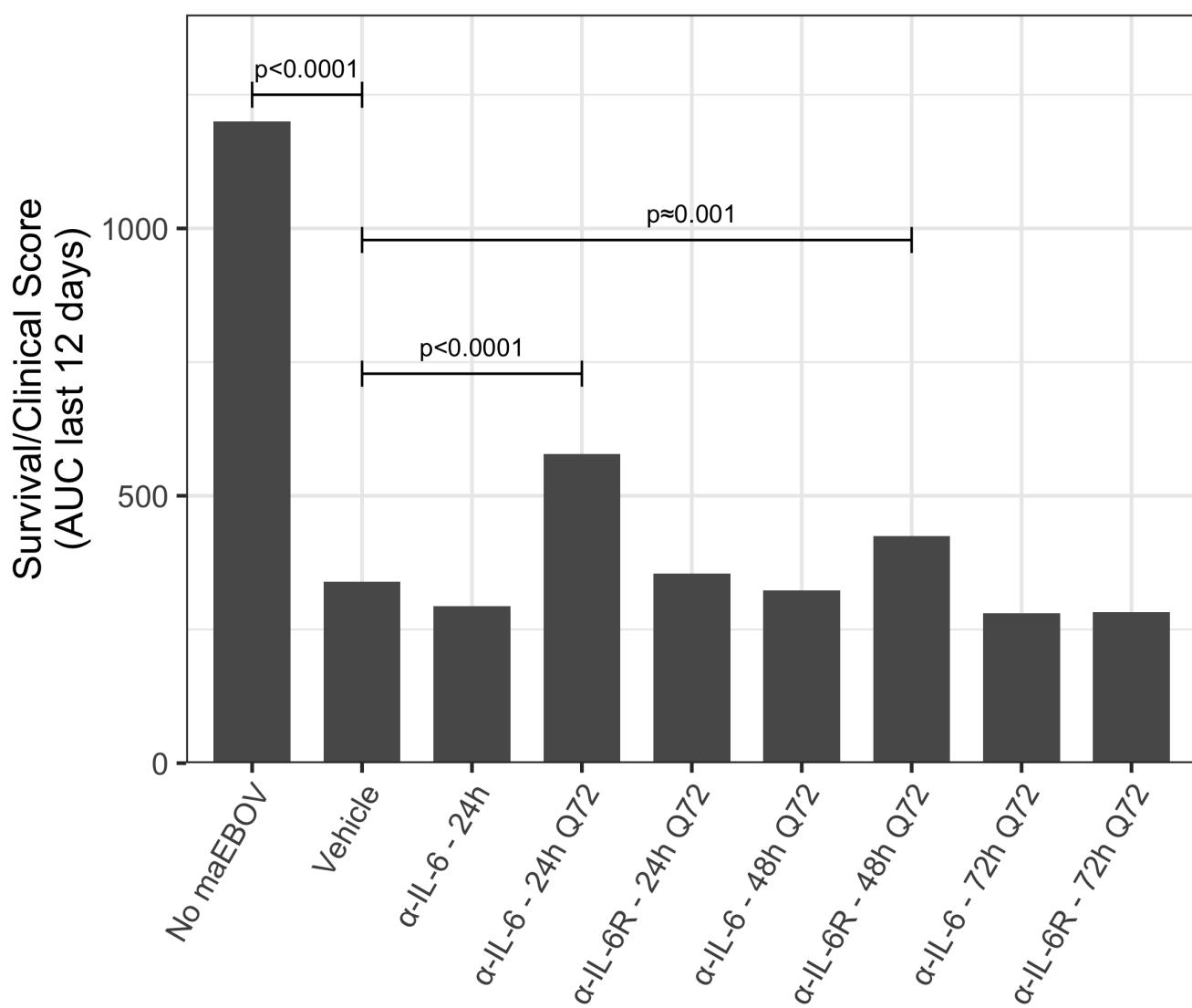


Figure 5. A clinical benefit metric was calculated as an area under curve for survival/clinical scores for 120 mice receiving a single or multiple i.p. doses of α -IL-6 or α -IL-6R mAb following maEBOV challenge on day 0. The given p values are determined from the Z statistic calculated for each experimental condition.

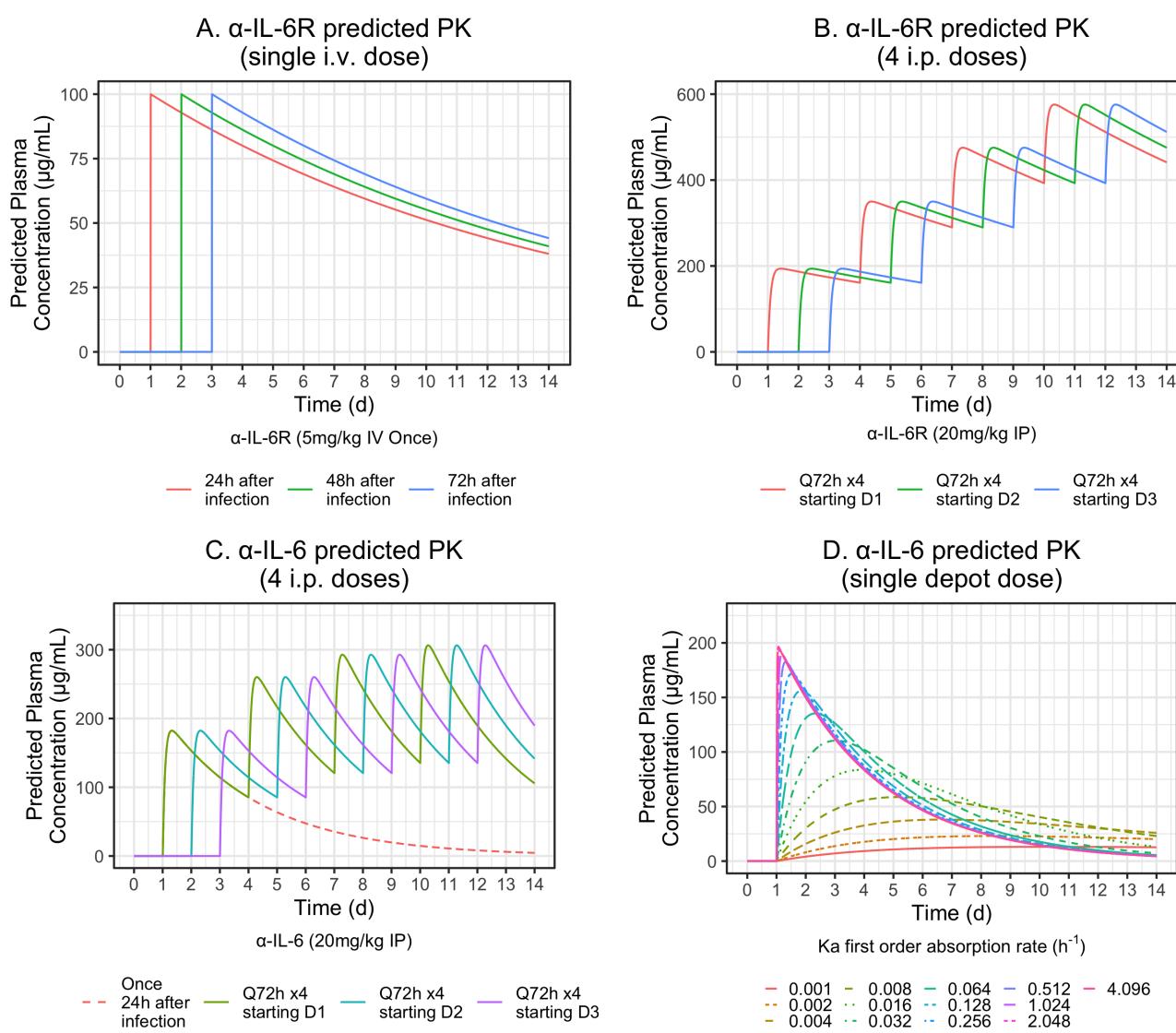


Figure 6. Simulated PK profiles for i.v. and i.p. routes of administration based on literature PK parameters shown in Table S5 in Supplemental Materials were determined. The top-left panel models the i.v. delivery experiment. The top-right and bottom-left panels model i.p. delivery experiments one and two. For each of these simulations, mice were dosed a total of four times at 72 hour intervals, beginning 24 hours after challenge. The bottom-right panel 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.