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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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 and coronavirus infection. However, IL-6 has also been shown to be an important mediator of innate immunity and 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 CRS. Most of these studies involve the use of anti-IL-6R monoclonal antibodies (α-IL-6R mAbs). We present data showing that direct neutralization of IL-6 with an α-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 α-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.

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 (CRS), commonly associated with Ebola virus disease (EVD) infections. (Wauquier et al., 2010). CRS is known to be a factor in morbidity and mortality associated with acute viral infections including those caused by filoviruses and coronaviruses. For example, non-survivors of the West African EBOV epidemics exhibited significantly elevated levels of the overall inflammatory response cytokines and monokines compared to survivors (Ruibal et al., 2016). It is thought that prolonged exposure to elevated inflamatory cytokine levels is toxic to T cells and results in their apoptotic and necrotic cell death (Younan et al., 2018). Both lymphopenia and elevated serum Il-6 levels are found in Ebola virus infection and are known to be inversely correlated with survival in patients post-infection (Wauquier et al., 2010) and in mouse models of Ebola infection (Herst et al., 2020). However, IL-6 has also been shown to be an important mediator of innate immunity and important for the host recovery from acute viral infection (Yang et al., 2017). Elevated IL-6 levels are also observed in SARS-CoV-2 infections, severe influenza , rhinovirus , RSV infection, as well as in similar respiratory infections (Conti et al., 2020; Hayden et al., 1998; Tang et al., 2016; Kerrin et al., 2017). Originally developed for the treatment of arthritis, α-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). 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, but also with mAb directed to IL-6 itself. We report here on the observed differences between treatments with α-IL-6R mAbs and α-IL-6 mAbs in a mouse model of EBOV infection 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) (Ebola virus M. musculus/COD/1976/Mayinga-CDC-808012), derived from the 1976 Zaire ebolavirus isolate Yambuku-Mayinga (Genebank accession NC002549), 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 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

rat anti-mouse-IL-6 IgG1 monoclonal antibody (BioXCell, BE0046, Lebanon, NH, RRID AB1107709) or rat anti-mouse-IL-6R IgG2 monoclonal antibody (BioXCell, BE0047, RRID AB1107588) at a dose of 100 μg in sterile saline via intravenous (i.v.) administration via an indwelling central venous catheter, or 400 μg via i.p. injection at 24, 48, or 72 hours post-challenge. Antibody dosing was based on amounts previously reported to neutralize IL-6 and IL-6R in mice (Liang et al., 2015; DL et al., 2014). 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 and Tables S2-S5 (Supplemental Materials). Control mice (n=36) were challenge with maEBOV in parallel, but were treated with antibody vehicle alone. Serum IL-6 measurements were performed in control rodents at necropsy as previously described (Herst et al., 2020).

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

The survival patterns for i.v. mAb treated and untreated groups following maEBOV challenge were statistically different and most untreated mice succumbed to maEBOV infection by day seven(Figure S1 , Supplementary Materials). 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 (see Figure S1, Panel C, Supplemental Materials). The Z statistic and significance level for this metric was calculated for each experimental condition. We found a minor clinical benefit (P <0.01) when mice were given one 100 ug dose of α-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 (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 toll-like receptor activation, we next compared the activities of α-IL-6 and α-IL-6R mAbs administered intraperitoneally following maEBOV challenge (Figures 2, 3, 4, and 5). We observed significant differences in the AUC Survival/Clinical Score when α-IL-6R mAb was administered 48 hours post maEBOV challenge and then repeated three times at 72 hour intervals. The most significant beneficial effect on the AUC Survival/Clinical Score (Figure 5) was seen when α-IL-6 mAb was administered beginning at 24 hours post maEBOV challenge, and then repeated three times at 72 hour intervals.

4 DISCUSSION

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

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

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

later α-IL-6R mAb is warranted to evaluate the potential of IL-6 pathway blockade in the during Ebola or 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 6. Dosing α-IL-6 mAb at 24 hours after challenge produced a clinical benefit, whereas dosing α-IL-6R beginning at the same time point did not. The shorter terminal half-life of α-IL-6 mAb ($T_{1/2}$ = $57h$) *versus* α-IL-6R mAb ($T_{1/2}$ = $223h$), possibly due to isotype specific differnces in glycosylation (Cobb, 2019) may help explain why giving α-IL-6 mAb early after infection provided the most observed clinical benefit. As can be seen from the simulated PK profile in Figure 6 (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 α-IL-6R where the blood levels continue to increase over the study period. These differences seen in the simulated PK profiles may have allowed α-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 α-IL-6 blockade *versus* α-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 α-IL-6R mAb, and that this may yield a potential advantage of using α-IL-6 mAb to treat CRS brought about by a viral infection.

It may be possible to develop a controlled release formulation of α-IL-6 mAb to obtain a clinically beneficial effect from the administration of α-IL-6 mAb, α-IL-6R mAb, or a combination of both, after a single injection early during the course of SARS-CoV-2 infection. For example, Figure 6, bottom-right panel, shows various predicted controlled release PK profiles of α-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.

5 CONCLUDING REMARKS

Although the previous reports of use of IL-6 blockers to treat CRS have shown mixed results, recent clinical data for α-IL-6 and α-IL-6R mAbs have shown early promise in human trials for treatment of severe influenza and corona virus infections (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 example, early α-IL-6 mAb and later α-IL-6R mAb, for the treatment of patients with CRS 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.

FUNDING

This study was funded by Flow Pharma, Inc. which had no influence over the content of this manuscript or the decision to publish.

REFERENCES

Ascierto, P. A., Fox, B. A., Urba, W. J., Anderson, A. C., Atkins, M. B., Borden, E. C., et al. (2020). Insights from immuno-oncology: the Society for Immunotherapy of Cancer Statement on access to IL-6-targeting therapies for COVID-19. *Journal for ImmunoTherapy of Cancer* 8, e000878. doi:10.1136/jitc-2020-000878

Biran, N., Ip, A., Ahn, J., Go, R. C., Wang, S., Mathura, S., et al. (2020). Tocilizumab among patients with covid-19 in the intensive care unit: a multicentre observational study. *The Lancet Rheumatology* doi:https://doi.org/10.1016/S2665-9913(20)30277-0

Bray, M., Davis, K., Geisbert, T., Schmaljohn, C., and Huggins, J. (1998). A Mouse Model for Evaluation of Prophylaxis and Therapy of Ebola Hemorrhagic Fever. *The Journal of Infectious Diseases* 178, 651–661. doi:10.1086/515386

Cassado, A. d. A., D'Império Lima, M. R., and Bortoluci, K. R. (2015). Revisiting Mouse Peritoneal Macrophages: Heterogeneity, Development, and Function. *Frontiers in Immunology* 6, 225. doi:10.3389/fimmu.2015.00225

Chan, M., Leung, A., Griffin, B. D., Vendramelli, R., Tailor, N., Tierney, K., et al. (2019). Generation and Characterization of a Mouse-Adapted Makona Variant of Ebola Virus. *Viruses* 11, 987–995. doi:10.3390/v11110987

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet* 395, 507–513. doi:10.1016/s0140-6736(20)30211-7

ClinicalTrialsEUSA (2020). EUSA Pharma initiates study of siltuximab to treat Covid-19 patients. *Clinical Trials Arena*

ClinicalTrialsGenetech (2020). Genentech's arthritis drug tocilizumab shows promise in Covid-19 trial. *Clinical Trials Arena*

Cobb, B. A. (2019). The history of IgG glycosylation and where we are now. *Glycobiology* 30, 202–213. doi:10.1093/glycob/cwz065

Comer, J., Escaffre, O., Neef, N., Brasel, T., Juelich, T., Smith, J., et al. (2019). Filovirus Virulence in Interferon α/β and γ Double Knockout Mice, and Treatment with Favipiravir. *Viruses* 11, 137. doi:10.3390/v11020137

Conti, P., Ronconi, G., Caraffa, A., Gallenga, C., Ross, R., Frydas, I., et al. (2020). Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *Journal of biological regulators and homeostatic agents* 34. doi:10.23812/conti-e

Della-Torre, E., Campochiaro, C., Cavalli, G., De Luca, G., Napolitano, A., La Marca, S., et al. (2020). Interleukin-6 blockade with sarilumab in severe covid-19 pneumonia with systemic hyperinflammation: an open-label cohort study. *Annals of the Rheumatic Diseases* doi:10.1136/annrheumdis-2020-218122

Dienz, O. and Rincon, M. (2009). The effects of IL-6 on CD4 T cell responses. *Clinical Immunology* 130, 27–33. doi:10.1016/j.clim.2008.08.018

DL, B., BB, A., C, M., I, S., and A, S. (2014). Role of IL-6 in Mycobacterium avium-associated immune reconstitution inflammatory syndrome. *J Immunol* 192, 676–682

EUSA (2015). Drug Approval Package: SYLVANT (siltuximab). *U.S. Food & Drug Administration*

Genentech (2014). Drug Approval Package: Actemra (tocilizumab) Solution for Subcutaneous Injection. *U.S. Food & Drug Administration*

Gritti, G., Raimondi, F., Ripamonti, D., Riva, I., Landi, F., Alborghetti, L., et al. (2020). Use of siltuximab in patients with COVID-19 pneumonia requiring ventilatory support. *medRxiv*

Guaraldi, G., Meschiari, M., Cozzi-Lepri, A., Milic, J., Tonelli, R., Menozzi, M., et al. (2020). Tocilizumab in patients with severe covid-19: a retrospective cohort study. *The Lancet Rheumatology* 2, e474 – e484. doi:https://doi.org/10.1016/S2665-9913(20)30173-9

Hayden, F., Fritz, R., Lobo, M., Alvord, W., Strober, W., and Straus, S. (1998). Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. *J Clin Invest.* 101, 643–649

Herper, M. (2020). Closely watched arthritis drug disappoints as a Covid-19 treatment, studies show. *STAT*

Herst, C., Burkholz, S., Sidney, J., Sette, A., Harris, P., Massey, S., et al. (2020). An effective CTL peptide vaccine for Ebola Zaire Based on Survivors' CD8+ targeting of a particular nucleocapsid protein epitope with potential implications for COVID-19 vaccine design. *Vaccine* doi:https://doi.org/10.1016/j.vaccine.2020.04.034

Hodge, T., Draper, K., Brasel, T., Freiberg, A., Squiquera, L., Sidransky, D., et al. (2016). Antiviral effect of ranpirnase against Ebola virus. *Antiviral Research* 132, 210–218. doi:10.1016/j.antiviral.2016.06.009

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020a). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet* 395, 497–506. doi:10.1016/s0140-6736(20)30183-5

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020b). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet* 395, 497 – 506. doi:https://doi.org/10.1016/S0140-6736(20)30183-5

Kaempf, J. W., Wang, L., and Dunn, M. (2019). Using a composite morbidity score and cultural survey to explore characteristics of high proficiency neonatal intensive care units. *Archives of Disease in Childhood - Fetal and Neonatal Edition* 104, F13–F17. doi:10.1136/archdischild-2017-313715

Kerrin, A., Fitch, P., Errington, C., Kerr, D., Waxman, L., Riding, K., et al. (2017). Differential lower airway dendritic cell patterns may reveal distinct endotypes of rsv bronchiolitis. *Thorax* 72, 620–627

Klopfenstein, T., Zayet, S., Lohse, A., Balblanc, J.-C., Badie, J., Royer, P.-Y., et al. (2020). Tocilizumab therapy reduced intensive care unit admissions and/or mortality in covid-19 patients. *Médecine et Maladies Infectieuses* 50, 397 – 400. doi:https://doi.org/10.1016/j.medmal.2020.05.001

Korn, T., Mitsdoerffer, M., Croxford, A. L., Awasthi, A., Dardalhon, V. A., Galileos, G., et al. (2008). IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. *PNAS; Proceedings of the National Academy of Sciences* 105, 18460–18465

Lane, T. R., Massey, C., Comer, J. E., Anantpadma, M., Freundlich, J. S., Davey, R. A., et al. (2019). Repurposing the antimalarial pyronaridine tetraphosphate to protect against Ebola virus infection. *PLOS Neglected Tropical Diseases* 13, 1–26. doi:10.1371/journal.pntd.0007890

Lee, D. W., Gardner, R., Porter, D. L., Louis, C. U., Ahmed, N., Jensen, M., et al. (2014). Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 124, 188–195. doi:10.1182/

blood-2014-05-552729

Lescure, F.-X., Bouadma, L., Nguyen, D., Parisey, M., Wicky, P.-H., Behillil, S., et al. (2020). Clinical and virological data of the first cases of COVID-19 in Europe: a case series. *The Lancet Infectious Diseases* doi:10.1016/s1473-3099(20)30200-0

Liang, Y., K, Y., J, G., J, W., YX, F., and H, P. (2015). Innate lymphotoxin receptor mediated signaling promotes HSV-1 associated neuroinflammation and viral replication. *Sci Reports* 5, 10406

Liu, B., Li, M., Zhou, Z., Guan, X., and Xiang, Y. (2020). Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? *Journal of Autoimmunity* , 102452doi:10.1016/j.jaut.2020.102452

Luo, P., Liu, Y., Qiu, L., Liu, X., Liu, D., and Li, J. (2020). Tocilizumab treatment in covid-19: A single center experience. *Journal of Medical Virology* 92, 814–818. doi:10.1002/jmv.25801

Medesan, C., Cianga, P., Mummert, M., Stanescu, D., Ghetie, V., and Ward, E. S. (1998). Comparative studies of rat IgG to further delineate the Fc:FcRn interaction site. *European Journal of Immunology* 28, 2092–2100. doi:10.1002/(sici)1521-4141(199807)28:07⟨2092::aid-immu2092⟩3.0.co;2-e

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., and Manson, J. J. (2020a). COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet* 395, 1033 – 1034. doi:https://doi.org/10.1016/S0140-6736(20)30628-0

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., and Manson, J. J. (2020b). COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet* 395, 1033–1034. doi:10.1016/s0140-6736(20)30628-0

Montesarchio, V., Parella, R., Iommelli, C., Bianco, A., Manzillo, E., Fraganza, F., et al. (2020). Outcomes and biomarker analyses among patients with covid-19 treated with interleukin 6 (il-6) receptor antagonist sarilumab at a single institution in italy. *Journal for ImmunoTherapy of Cancer* 8. doi:10.1136/jitc-2020-001089

Price, C. C., Altice, F. L., Shyr, Y., Koff, A., Pischel, L., Goshua, G., et al. (2020). Tocilizumab treatment for cytokine release syndrome in hospitalized covid-19 patients: Survival and clinical outcomes. *Chest* doi:https://doi.org/10.1016/j.chest.2020.06.006

Ruan, Q., Yang, K., Wang, W., Jiang, L., and Song, J. (2020). Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Medicine* 46, 846–848. doi:10.1007/s00134-020-05991-x

Ruibal, P., Oestereich, L., Lüdtke, A., Becker-Ziaja, B., Wozniak, D. M., Kerber, R., et al. (2016). Unique human immune signature of Ebola virus disease in Guinea. *Nature* 533, 100–104

Saha, A., Sharma, A. R., Bhattacharya, M., Sharma, G., Lee, S.-S., and Chakraborty, C. (2020). Tocilizumab: A Therapeutic Option for the Treatment of Cytokine Storm Syndrome in COVID-19. *Archives of Medical Research* doi:https://doi.org/10.1016/j.arcmed.2020.05.009

Sanofi (2017). Drug Approval Package: Kevzara (sarilumab) Injection. *U.S. Food & Drug Administration*

Sciascia, S., Apr?, F., Baffa, A., Baldovino, S., Boaro, D., Boero, R., et al. (2020). Pilot prospective open, single-arm multicentre study on off-label use of tocilizumab in patients with severe COVID-19. *Clin. Exp. Rheumatol.* 38, 529–532

Sinha, P., Mostaghim, A., Bielick, C. G., McLaughlin, A., Hamer, D. H., Wetzler, L. M., et al. (2020). Early administration of interleukin-6 inhibitors for patients with severe covid-19 disease is associated with decreased intubation, reduced mortality, and increased discharge. *International Journal of Infectious Diseases* 99, 28 – 33. doi:https://doi.org/10.1016/j.ijid.2020.07.023

Somers, E. C., Eschenauer, G. A., Troost, J. P., Golob, J. L., Gandhi, T. N., Wang, L., et al. (2020). Tocilizumab for treatment of mechanically ventilated patients with COVID-19. *Clinical Infectious*

Diseases doi:10.1093/cid/ciaa954. Ciaa954

Swaak, A. J., Rooyen, A. V., Nieuwenhuis, E., and Aarden, L. A. (1988). lnterleukin-6 (IL-6) in Synovial Fluid and Serum of Patients with Rheumatic Diseases. *Scandinavian Journal of Rheumatology* 17, 469–474. doi:10.3109/03009748809098809

Tan, L., Wang, Q., Zhang, D., Ding, J., Huang, Q., Tang, Y., et al. (2020). Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct Target Ther.* 5, 33

Tanaka, T., Narazaki, M., and Kishimoto, T. (2016). Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy* 8, 959–970. doi:10.2217/imt-2016-0020

Tang, F., Hansbro, P., Burgess, J., Ammit, A., Baines, K., and Oliver, B. (2016). A novel immunomodulatory function of neutrophils on rhinovirus-activated monocytes in vitro. *Thorax* 71, 1039–1049

Taylor, P. (2020). Novartis to test canakinumab for COVID-19, as IL-6 trial disappoints. *PMLiVE*

Toniati, P., Piva, S., Cattalini, M., Garrafa, E., Regola, F., Castelli, F., et al. (2020). Tocilizumab for the treatment of severe covid-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in brescia, italy. *Autoimmunity Reviews* 19, 102568. doi:https://doi.org/10.1016/j.autrev.2020.102568. Special issue COVID19 and Autoimmunity

Vanoni, S., Tsai, Y.-T., Waddell, A., Waggoner, L., Klarquist, J., Divanovic, S., et al. (2017). Myeloid-derived NF-$\kappa\beta$ negative regulation of PU.1 and c/EBP-β-driven pro-inflammatory cytokine production restrains LPS-induced shock. *Innate Immunity* 23, 175–187. doi:10.1177/1753425916681444. PMID: 27932520

Wauquier, N., Becquart, P., Padilla, C., Baize, S., and Leroy, E. M. (2010). Human fatal zaire ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis. *PLoS Negl. Trop. Dis.* 4. doi:10.1371/journal.pntd.0000837

Wong, C. K., Lam, C. W. K., Wu, A. K. L., Ip, W. K., Lee, N. L. S., Chan, I. H. S., et al. (2004). Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clinical & Experimental Immunology* 136, 95–103. doi:10.1111/j.1365-2249.2004.02415.x

Xu, X., Han, M., Li, T., Sun, W., Wang, D., Fu, B., et al. (2020). Effective treatment of severe COVID-19 patients with tocilizumab. *PNAS; Proceedings of the National Academy of Sciences* 117, 10970–10975

Yang, M., Wang, C., Yang, S., Leu, C., Chen, S., Wu, C., et al. (2017). IL-6 ameliorates acute lung injury in influenza virus infection. *Sci Reports* 7, 43829

Younan, P., Iampietro, M., and Bukreyev, A. (2018). Disabling of lymphocyte immune response by Ebola virus. *PLoS Pathog* 14, e1006932

Younan, P., Santos, R., Ramanathan, P., Iampietro, M., Nishida, A., Dutta, M., et al. (2019). Ebola virus-mediated T-lymphocyte depletion is the result of an abortive infection. *PLoS Pathog.* 15, e1008068

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet* 395, 1054–1062. doi:10.1016/s0140-6736(20)30566-3

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, SpO2<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, SpO2<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, SpO2<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-Reative protein levels relative to pretreatment levels Luo et al. (2020)
RT-PCR confirmed Sars Cov-2 pneumonia, Sp02<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 snd X-ray confirmed Sars Cov-2 pneumonia, Sp02<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 adminstration of Tocilizumab (p<0.002). Klopfenstein et al. (2020)
RT-PCR confirmed Sars Cov-2 pneumonia, SpO2<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, SpO2<92% in room air	PROSPECTIVE SINGLE ARM: Pre- and Post-Tocilizumab (n=63) OUTCOME: Clinical parameters (CRP levels and ratio PaO2/FiO2)	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, SpO2<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, SpO2<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. SpO2<92%	PROSPECTIVE SINGLE ARM with two subgroups (A (n=149): requiring FiO2<45% and B (n=106): requiring FiO2>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)

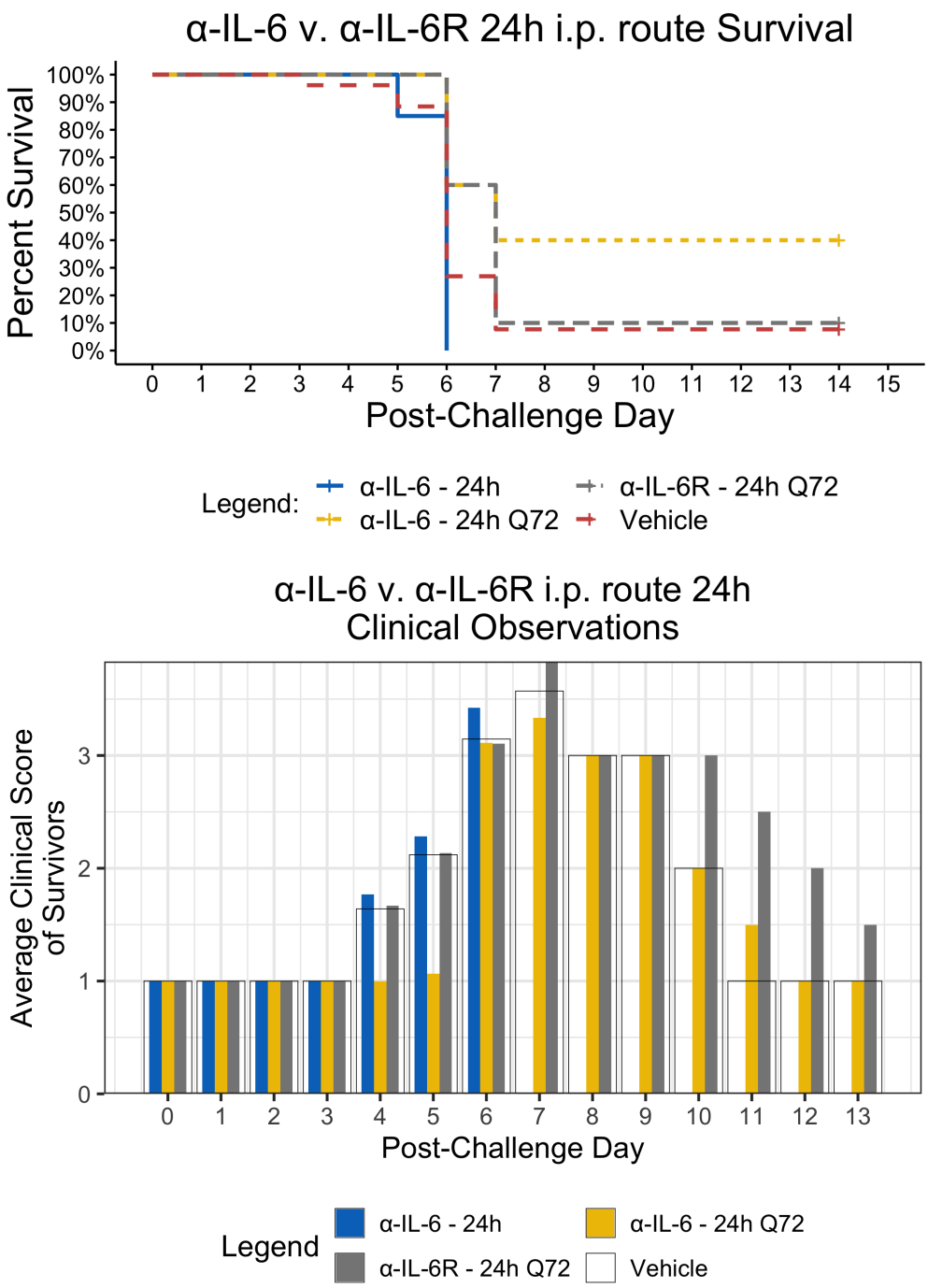

Figure 2. Kaplan-Meier Survival Plots and Average clinical scores for a single or multiple i.p. doses of α-IL-6 or α-IL-6R administered 24 hours after maEBOV challenge and followed by repeat dosing every 72 hours for a total of four doses. The survival curves were significantly different by Log-rank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

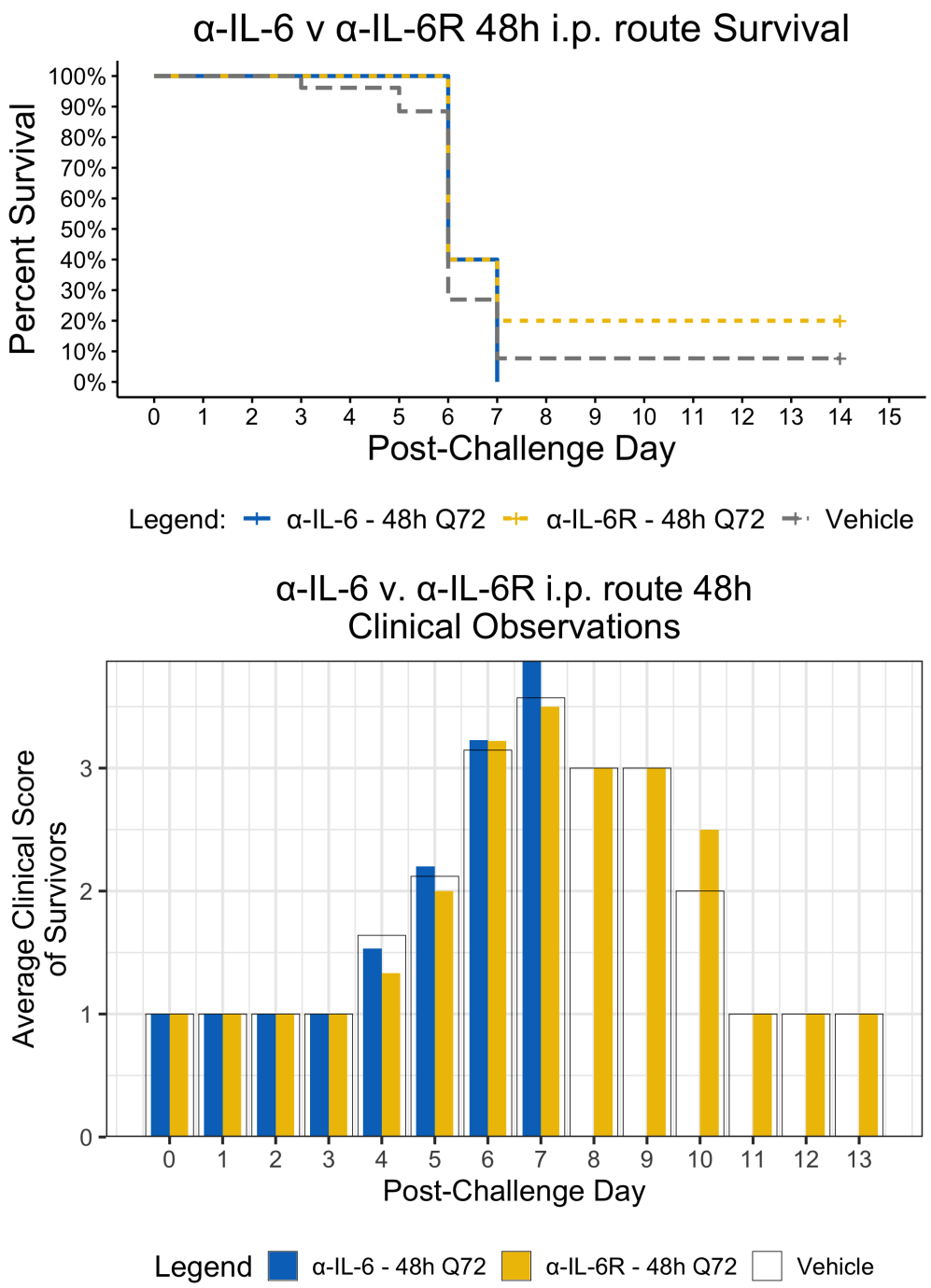

Figure 3. Kaplan-Meier Survival Plots and Average clinical scores for multiple i.p. doses of α-IL-6 or α-IL-6R administered 48 hours after maEBOV challenge and followed by repeat dosing every 72 hours for a total of four doses. The survival curves were significantly different by Log-rank (Mantel-Cox)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

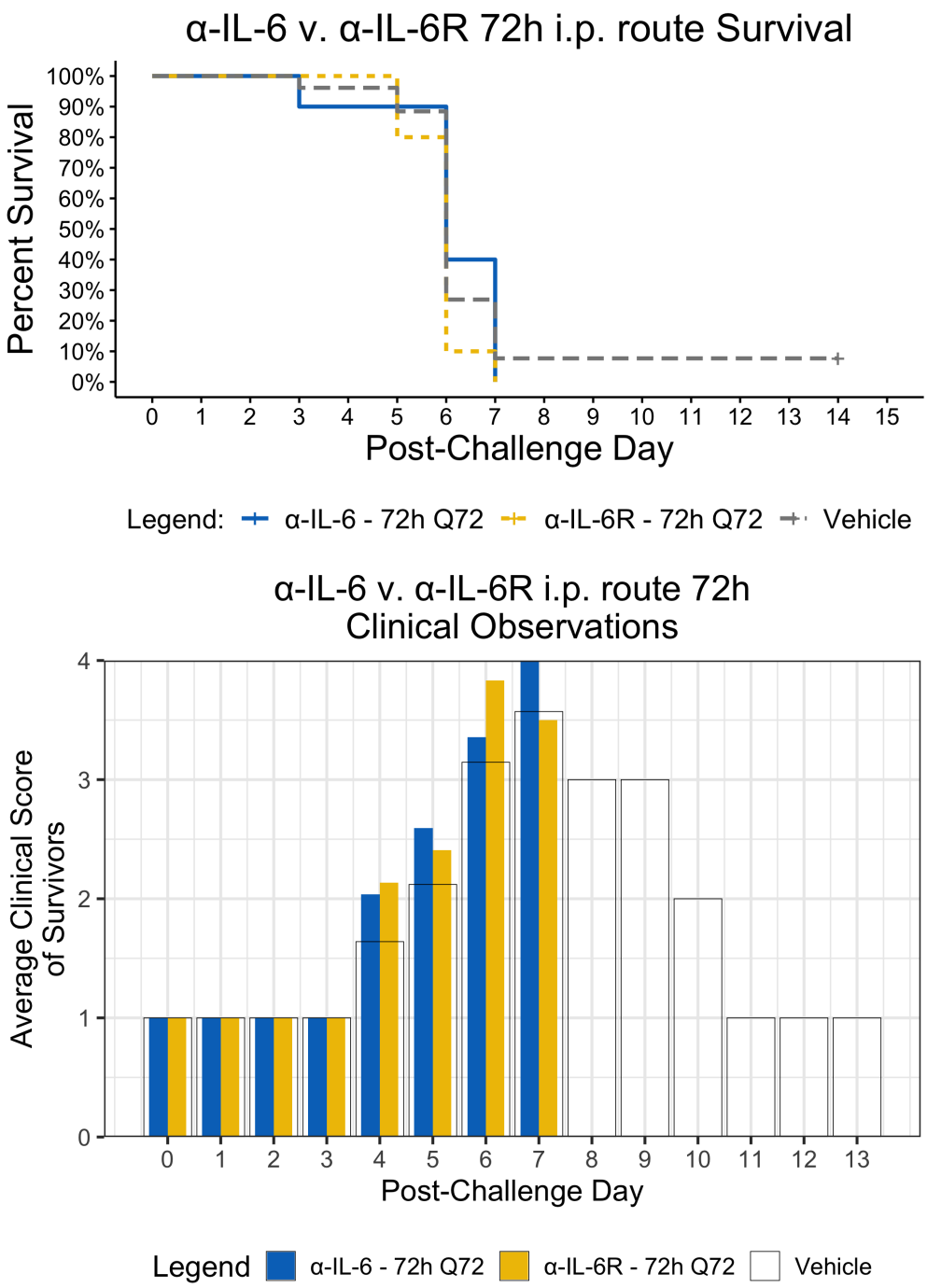

Figure 4. Kaplan-Meier Survival Plots and Average clinical scores for multiple i.p. doses of α-IL-6 or α-IL-6R administered 72 hours after maEBOV challenge and followed by repeat dosing every 72 hours for a total of four doses. The survival curves were significantly different by Log-rank (Mantel-Cox)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

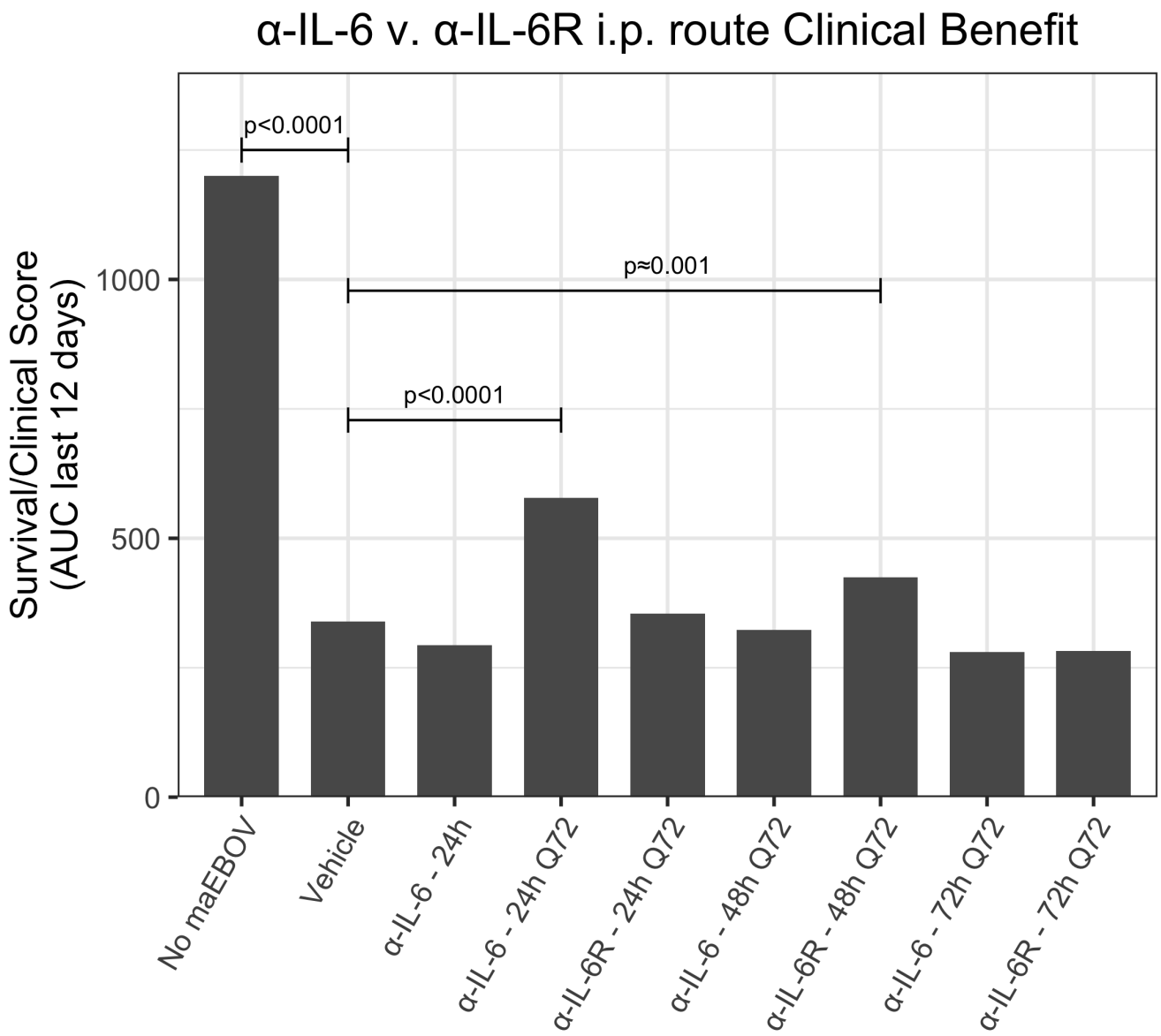

Figure 5. A clinical benefit metric was calculated as an area under curve for survival/clinical scores for 120 mice receiving a single or multiple i.p. doses of α-IL-6 or α-IL-6R mAb following maEBOV challenge on day 0. The given p values are determined from the Z statistic calculated for each experimental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

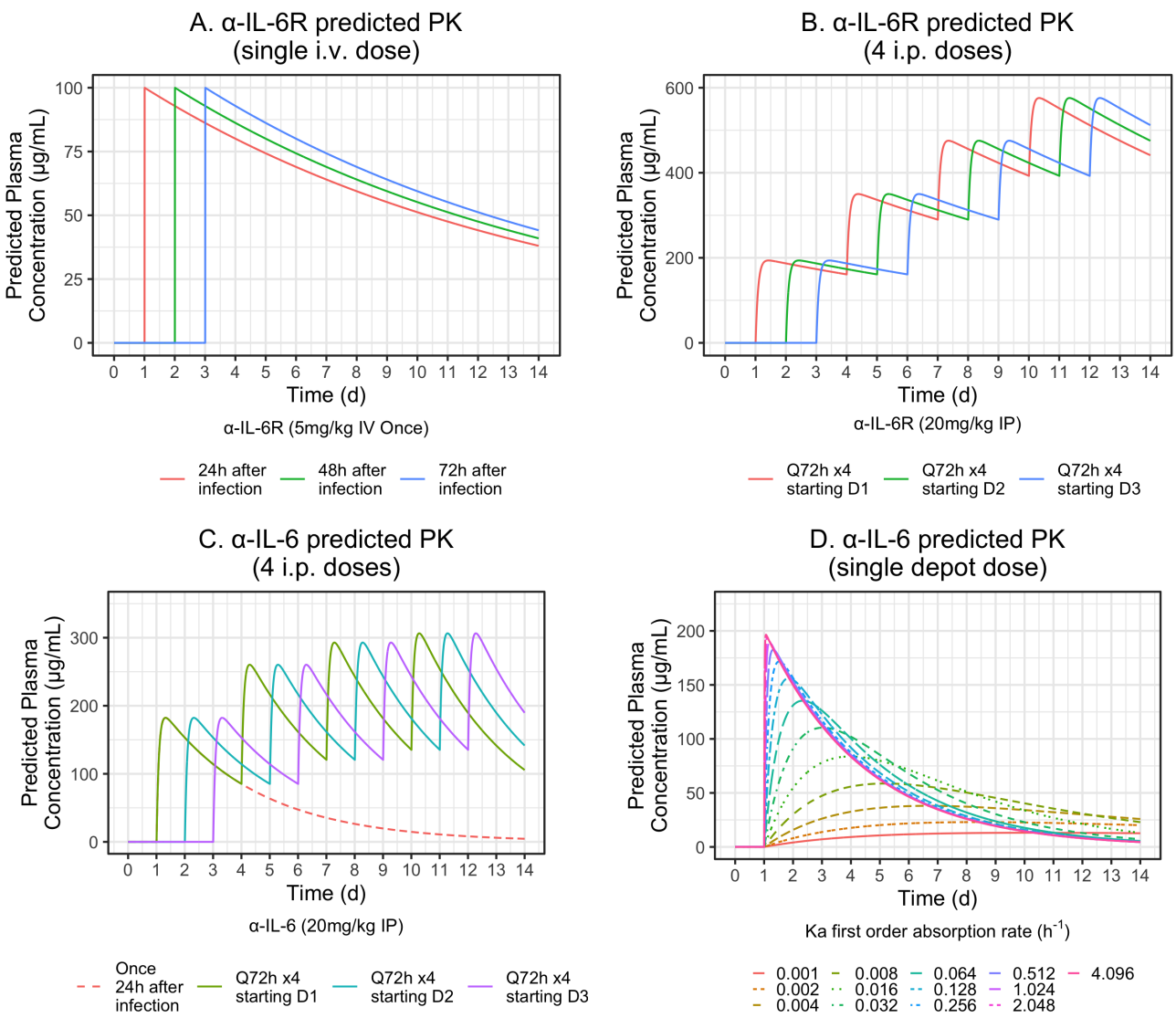

Figure 6. Simulated PK profiles for i.v. and i.p. routes of administration based on literature PK parameters shown in Table S5 in Supplemental Materials were determined. The top-left panel models the i.v. delivery experiment. The top-right and bottom-left panels model i.p. delivery experiments one and 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.