Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Antiviral Research 163 (2019) 70–74

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Prophylactic efficacy of a human monoclonal antibody against MERS-CoV in the common marmoset

Emmie de Wit[a], Friederike Feldmann[b], Eva Horne[a], Atsushi Okumura[c], Elisabetta Cameroni[d], Elaine Haddock[a], Greg Saturday[b], Dana Scott[b], Robin Gopal[e], Maria Zambon[e], Davide Corti[d], Heinz Feldmann[a,*]

[a] Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA
[b] Rocky Mountain Veterinary Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA
[c] Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, USA
[d] Humabs BioMed SA, A Subsidiary of Vir Biotechnology, 6500, Bellinzona, Switzerland
[e] National Infection Service, Public Health England (PHE), London, NW9 5EQ, United Kingdom

ARTICLE INFO

Keywords:
MERS-CoV
Treatment
Prophylaxis
Neutralizing monoclonal antibody
LCA60
Common marmoset

ABSTRACT

Effective antiviral treatments for MERS-CoV are urgently needed. LCA60 is a MERS-CoV-neutralizing monoclonal antibody isolated from a convalescent MERS patient. Previously, it was shown that treatment with LCA60 resulted in reduced disease and virus titers in mouse models of MERS-CoV infection. Here, we tested the prophylactic efficacy of LCA60 in the common marmoset model of MERS-CoV infection. Intravenous administration of LCA60 one day before virus challenge resulted in high levels of MERS-CoV-neutralizing activity in circulating blood. Clinically, there was a moderate benefit of treatment with LCA60 including reduced respiratory involvement. Although viral lung loads were not reduced in LCA60-treated animals as compared to controls, there were fewer pathological changes in the lungs. Thus, prophylactic LCA60 treatment could be implemented to reduce disease burden in contacts of confirmed MERS-CoV patients.

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) can cause severe respiratory disease with a high case-fatality rate in humans. There are currently no approved treatments for MERS. LCA60 is a human IgG1 monoclonal antibody that was isolated from the memory B cells derived from a convalescent MERS patient in the UK (Bermingham et al., 2012; Corti et al., 2016) that neutralizes MERS-CoV by binding to the receptor binding domain of the MERS-CoV spike protein (Corti et al., 2015). Balb/c mice transiently expressing human DPP4 in lung tissue after transduction with an adenovirus vector expressing human DPP4 and treated with LCA60 one day before or after challenge with MERS-CoV showed a significant reduction of virus titers in the lungs compared to controls (Corti et al., 2015). In hDPP4-transduced IFNAR-KO mice, treatment with LCA60 one day after inoculation with MERS-CoV resulted in reduced disease, as indicated by bodyweight loss, as well as a significant reduction in lung virus titers (Corti et al., 2015). These promising results prompted us to test the prophylactic efficacy of LCA60 treatment in a distinct animal model of MERS, the common marmoset, to support licensure for human use. In contrast to mice, common marmosets are naturally susceptible to MERS-CoV and develop moderate to severe disease upon infection (Falzarano et al., 2014).

All animal experiments were approved by the Institutional Animal Care and Use Committee of Rocky Mountain Laboratories, NIAID, NIH and carried out by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited facility, according to the institution's guidelines for animal use, and followed the guidelines and basic principles in the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Guide for the Care and Use of Laboratory Animals. All infectious work with MERS-CoV was approved by the Institutional Biosafety Committee and performed in a high containment facility at RML. Sample inactivation was performed according to standard operating procedures for removal of specimens from high containment approved by the Institutional Biosafety Committee.

Twelve common marmosets (Callithrix jacchus; 2–6 years old) were randomly assigned into two groups of six animals. Animals in the first group (5 males; 1 female) were intravenously infused with 30 mg/kg of mAb LCA60; those in the second group (4 males, 2 females) were intravenously infused with MPE8, a human monoclonal antibody that neutralizes human respiratory syncytial virus (hRSV) and was used as a

* Corresponding author. 903 South 4th Street, Hamilton, MT, 59840, USA.
E-mail address: feldmannh@niaid.nih.gov (H. Feldmann).

https://doi.org/10.1016/j.antiviral.2019.01.016
Received 21 January 2019; Accepted 23 January 2019
Available online 24 January 2019
0166-3542/ Published by Elsevier B.V.

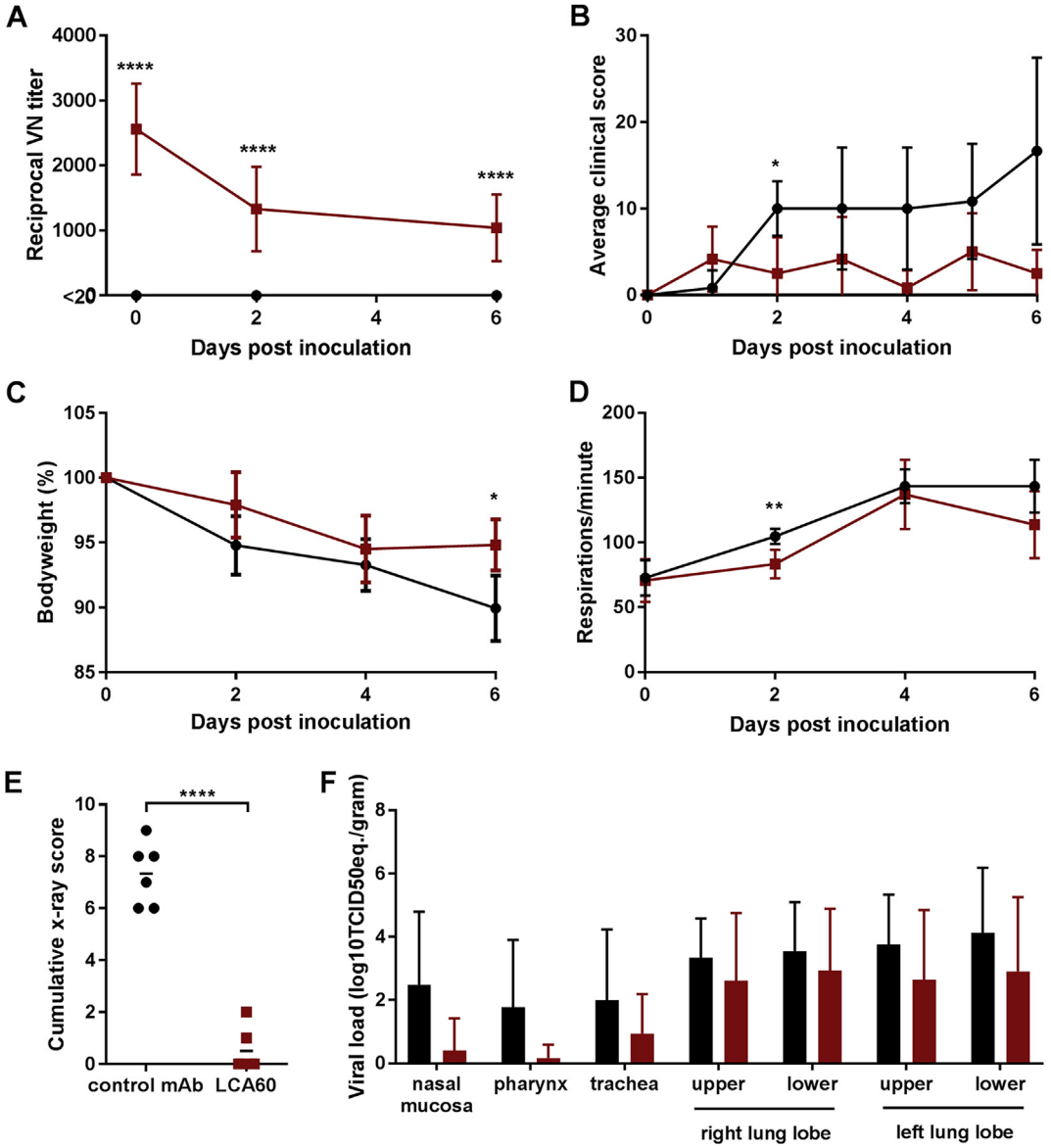

Fig. 1. Serological, clinical and virological findings in common marmosets inoculated with MERS-CoV (strain hCoV-EMC/2012) and treated prophylactically with LCA60. Two groups of six marmosets were intravenously infused with 30 mg/kg control mAb (black circles) or MERS-CoV neutralizing mAb LCA60 (red squares) and inoculated with MERS-CoV 24 h later. On 0, 2 and 6 dpi, serum was collected and tested for the presence of MERS-CoV neutralizing antibodies (A). After inoculation, the animals were observed twice daily for clinical signs of disease and scored using a clinical scoring system prepared for common marmosets by a person blinded to treatment assignment (B). On 0, 2, 4 and 6 dpi, clinical exams were performed during which bodyweight (C) and respiration rate (D) were determined and radiographs were taken. Radiographs were used to score individual lung lobes for severity of pulmonary infiltrates by a clinical veterinarian blinded to treatment assignment according to a standard scoring system (0: normal; 1: mild interstitial pulmonary infiltrates; 2: moderate pulmonary infiltrates perhaps with partial cardiac border effacement and small areas of pulmonary consolidation; 3: serious interstitial infiltrates, alveolar patterns and air bronchograms); the cumulative x-ray score is the sum of the scores of the four individual lung lobes per animal (E). On 6 dpi all animals were euthanized and tissue samples were collected from all 4 lung lobes, RNA was extracted, qRT-PCR targeting UpE performed, and viral load determined as $TCID_{50}$ equivalents per gram tissue as described previously (de Wit et al., 2018). Geometric mean viral loads and standard deviation are shown for each tissue (F). Asterisks indicate statistical significance determined using an unpaired *t*-test with multiple comparisons using the Holm-Sidak method; *: $p < 0.05$; **: $p < 0.01$; ****: $p < 0.0001$.

control (Corti et al., 2013). Monoclonal antibodies were infused into the femoral vein in a volume of ≤1 ml depending on bodyweight. MERS-CoV-neutralizing activity in serum was determined at several time-points after infusion using a microneutralization assay with 100 $TCID_{50}$ hCoV-EMC/2012 as described previously (de Wit et al., 2018). Serum samples collected on 1, 3 and 7 days after administration of LCA60 contained high levels of MERS-CoV neutralizing activity; no MERS-CoV neutralizing antibodies were detected in the control mAb-treated animals (Fig. 1A). Although neutralizing titers in the serum of LCA60-treated animals dropped between the first and last measurement, reciprocal serum neutralizing titers remained high throughout the experiment in all animals (range 480–1920; Fig. 1A). Twenty-four hours after administration of neutralizing antibody preparations, all animals were inoculated with MERS-CoV (strain hCoV-EMC/2012) by a combination of four routes with a total dose of dose 5.2×10^6 $TCID_{50}$ (intranasally with 100 µl in each nare, orally with 500 µl, intratracheally with 500 µl and in each eye with 50 µl of DMEM containing 4×10^6 $TCID_{50}$/ml) as established previously (Falzarano et al., 2014) to determine the prophylactic efficacy of mAb treatment. After inoculation with MERS-CoV, animals were scored twice daily for the presence of disease signs using a standardized scoring sheet previously developed and published for common marmosets (Falzarano et al.,

2014). Scoring was done by the same individual throughout the study; this person was blinded to assignment of the animals to the control mAb- or LCA60-treated group. Regardless of treatment, all animals showed signs of disease after inoculation with MERS-CoV; however, disease scores appeared lower in the animals treated with LCA60, although this difference was statistically significant only on 2 days post infection (dpi) (Fig. 1B). Clinical exams were performed on anesthetized animals on 0, 2, 4 and 6 dpi during which bodyweight and respiration rate were determined and chest radiographs were taken. All animals lost bodyweight after inoculation with MERS-CoV; on 6 dpi the bodyweight loss observed in the LCA60-treated animals was statistically significantly less than that in the control animals (Fig. 1C). The animals treated with control mAb all showed increased respiration rates whereas only 5 of 6 animals treated with LCA60 showed increased respiration rates. Respiration rates were significantly higher in the control mAb-treated animals than the LCA60-treated animals on 2 dpi, but not on 4 or 6 dpi (Fig. 1D). Chest radiographs obtained on 6 dpi from all animals were analyzed for the presence of pulmonary infiltrates by a clinical veterinarian blinded to the group assignment of the animals and showed that LCA60-treated animals had significantly lower cumulative scores than control mAb-treated animals (Fig. 1E), indicating that LCA60-treated animals had fewer pulmonary infiltrates and suggesting that respiratory disease was less severe in LCA60-treated animals. One of the common marmosets in the control group reached the pre-established humane endpoint criteria on 6 dpi, the final day of the experiment, with severe respiratory signs, a clinical score of 35 and severe hypothermia (body temperature 33.9 °C, 5.6 °C below baseline; data not shown).

On 6 dpi, all animals were euthanized to determine viral loads and assess pathologic changes in various tissues. Tissues of the respiratory tract, including samples of all four lung lobes, were collected for analysis. RNA was extracted from 30 mg tissue and used in a one-step real-time RT-PCR (qRT-PCR) detecting upE (Corman et al., 2012) as described previously (de Wit et al., 2018). There were no statistical differences in viral loads in LCA60-treated versus control mAb-treated animals (Fig. 1D). Viral loads were determined by qRT-PCR as virus titration in the presence of neutralizing antibody would be hard to interpret since neutralizing antibodies would still be present in the tissue when titration assays are performed, and previously unbound antibody could potentially neutralize virus during titration, making negative findings difficult to interpret. Upon necropsy, the area of lung lobes affected by gross lesions was estimated by a board-certified veterinary pathologist who was blinded to the group assignment of the animals. Although gross lung lesions were observed in all LCA60-and control mAb-treated animals, a statistically significantly smaller area of the lung was affected by lesions in LCA60-treated animals than control-mAb-treated animals (Fig. 2A). Lung weights were determined at necropsy to calculate the lung weight to bodyweight ratio as an indicator of pneumonia; this ratio was significantly lower in the LCA60-treated animals than in the controls (Fig. 2B). Histological assessment of lung tissue by a board-certified veterinary pathologist blinded to group assignment of the animals, showed that all animals except one animal in the LCA60-treated group developed the typical multifocal to coalescing, moderate to marked subacute bronchointerstitial pneumonia with type II pneumocyte hyperplasia (Fig. 2C) as previously observed in marmosets inoculated with MERS-CoV (Falzarano et al., 2014). MERS-CoV antigen could be detected by immunohistochemistry in areas affected by lesions using an in-house rabbit polyclonal antibody against MERS-CoV described elsewhere (de Wit et al., 2013) (Fig. 2C). Quantification of the percentage of MERS-CoV antigen-positive cells in lung histology slides, which was performed using an Aperio Digital Slide Scanner and the ImageScope positive pixel count algorithm as described previously (Baseler et al., 2016), showed that statistically significantly fewer antigen-positive cells were present in the lungs of LCA60-treated animals than in the lungs of control animals (Fig. 2D). In the one LCA60-treated animal without histological lesions, viral RNA was not detected in the lung lobes by qRT-PCR, but was detected in conjunctiva, pharynx and nasal mucosa. Whether the lack of lesions and virus replication in the lungs of this animal was a result of LCA60 treatment, sampling error or a problem with virus inoculation could not be determined.

Taken together, the prophylactic treatment with LCA60 resulted in a moderate clinical benefit, including reduced respiratory involvement and fewer pathological changes in the lungs of LCA60-treated marmosets. These results are in line with a study in common marmosets where treatment with monoclonal antibodies REGN3048 and REGN3051 resulted in less severe respiratory disease (de Wit et al., 2018), and a study in rhesus macaques where prophylactic treatment with the MERS-CoV neutralizing mAb 3B11−N resulted in a reduced pathologic lung volume using computed tomography (Johnson et al., 2016). Of note, LCA60 was shown to bind to an epitope overlapping with that of 3B11−N and to be 10-fold more potent in terms of viral neutralization (Corti et al., 2015). Thus, our study supports implementation of prophylactic LCA60 treatment to reduce health care center-based outbreaks and transmission to close contacts of confirmed MERS-CoV patients.

Therapeutic efficacy of LCA60 was not assessed in the marmoset model here due to the moderate clinical benefit observed upon prophylactic treatment, but has been shown in a mouse model of MERS-CoV infection (Corti et al., 2015). However, therapeutic efficacy of several MERS-CoV neutralizing antibodies has been shown in mouse models (Agrawal et al., 2016; Corti et al., 2015; Li et al., 2015; Pascal et al., 2015; Qiu et al., 2016; Zhao et al., 2018), but so far the clinical benefit in nonhuman primate studies has been modest (Chen et al., 2017; de Wit et al., 2018; van Doremalen et al., 2017). Thus, LCA60 would likely be more efficacious as a therapeutic treatment in combination with other MERS-CoV-neutralizing antibodies or other antiviral treatments such as ribavirin, IFN and lopinavir that have shown therapeutic efficacy in nonhuman primates (Chan et al., 2015; Falzarano et al., 2013). A limitation of this study is reflected by the debate on the value of animal disease models for MERS (reviewed in (Baseler et al., 2016)). Neither the mouse nor the nonhuman primate models represent all hallmarks of human MERS. Rodents lack natural susceptibility to MERS-CoV which is overcome by expressing the human receptor by different means. The nonhuman primate models utilize high infectious doses and partial artificial inoculation routes leading to immediate acute but transient disease that can be fatal in common marmosets. Currently, there are insufficient data to address the value of one model over the other (reviewed in (Baseler et al., 2016)). Therefore, treatment efficacy data in both rodent and nonhuman primate models need to be considered and interpreted carefully as they may not necessarily reflect the outcome in humans. Until further evaluation, both these models seem needed to select treatment modalities prior to human clinical trials and thus are important tools for MERS countermeasure development.

Conflicts of interest

EdW, FF, EvH, AO, ElH, GS, DS, RG, MZ and HF have no conflicts to declare.

EC and DC are employees of Vir Biotechnology and hold shares in Vir Biotechnology.

Acknowledgements

The authors would like to thank the members of the Rocky Mountain Veterinary Branch (DIR, NIAID, NIH) for their assistance with this study. This work was supported in part by the Intramural Research Program of NIAID, NIH and federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Department of Health and Human Services.

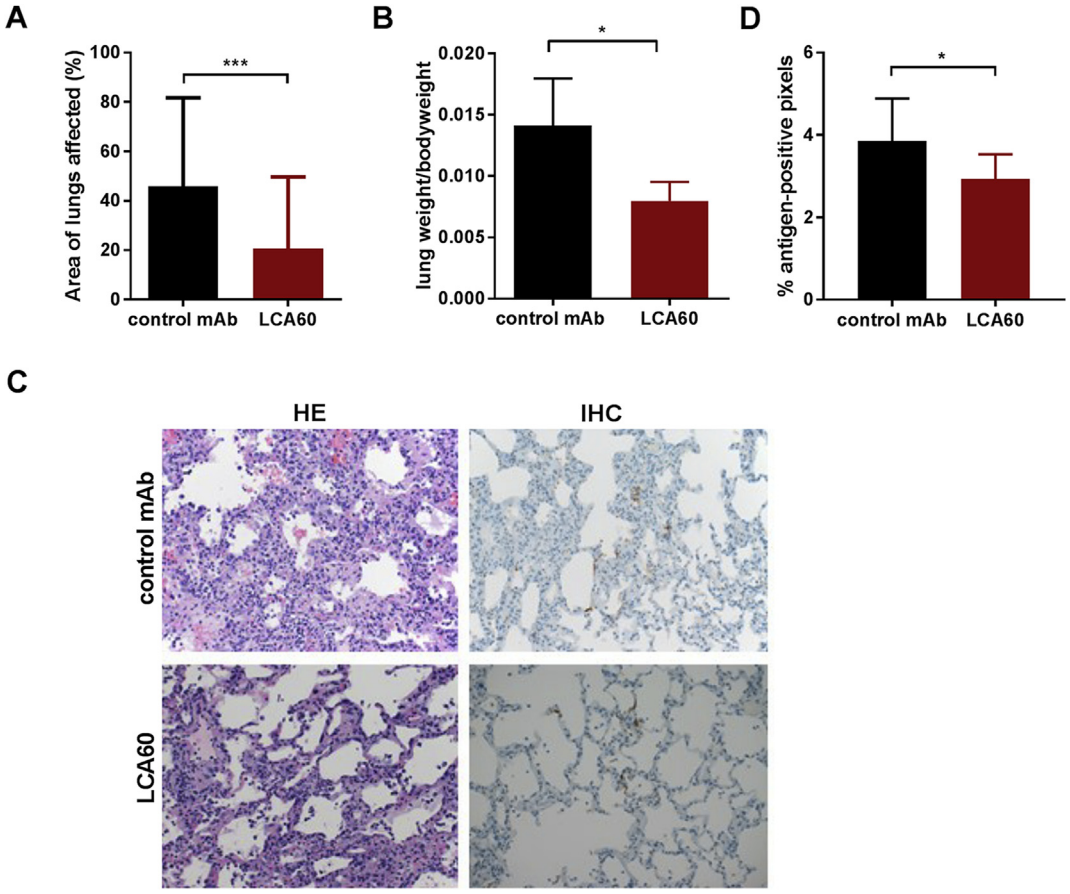

Fig. 2. Pathological findings in the lungs of common marmosets inoculated with MERS-CoV and treated prophylactically with LCA60. Two groups of six common marmosets were intravenously infused with 30 mg/kg control mAb (black) or MERS-CoV neutralizing mAb LCA60 (red) and inoculated with MERS-CoV 24 h later. On 6 dpi all animals were euthanized and the area of the lung lobes affected by gross lesions was scored by a board-certified veterinary pathologist blinded to treatment received by the animals (A); lungs were weighed to determine the lung weight:bodyweight ratio (B) and samples were collected for histological analysis. Tissues were stained with hematoxylin and eosin (HE) or a rabbit polyclonal α-MERS-CoV antibody (IHC). One representative image was chosen for each group of control mAb-treated and LCA60-treated animals (C). Slides of all four lung lobes of all animals stained with a polyclonal α-MERS-CoV antibody were digitized and antigen-positive pixels were quantified using the ImageScope positive pixel algorithm. The percentage antigen-positive pixels was calculated as the number of pixels stained for MERS-CoV antigen divided by the total number of stained pixels (i.e. non-stained areas such as air spaces were excluded from the analysis) (D). Asterisks indicate statistical significance determined using an unpaired *t*-test with multiple comparisons using the Holm-Sidak method; *: $p < 0.05$; ***: $p < 0.001$.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.01.016.

References

Agrawal, A.S., Ying, T., Tao, X., Garron, T., Algaissi, A., Wang, Y., Wang, L., Peng, B.H., Jiang, S., Dimitrov, D.S., Tseng, C.T., 2016. Passive transfer of A germline-like neutralizing human monoclonal antibody protects transgenic mice against lethal Middle East respiratory syndrome coronavirus infection. Sci. Rep. 6, 31629.

Baseler, L., de Wit, E., Feldmann, H., 2016. A comparative review of animal models of Middle East respiratory syndrome coronavirus infection. Vet. Pathol. 53, 521–531.

Bermingham, A., Chand, M.A., Brown, C.S., Aarons, E., Tong, C., Langrish, C., Hoschler, K., Brown, K., Galiano, M., Myers, R., Pebody, R.G., Green, H.K., Boddington, N.L., Gopal, R., Price, N., Newsholme, W., Drosten, C., Fouchier, R.A., Zambon, M., 2012. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill. bulletin europeen sur les maladies transmissibles = European communicable disease bulletin 17, 20290.

Chan, J.F., Yao, Y., Yeung, M.L., Deng, W., Bao, L., Jia, L., Li, F., Xiao, C., Gao, H., Yu, P., Cai, J.P., Chu, H., Zhou, J., Chen, H., Qin, C., Yuen, K.Y., 2015. Treatment with lopinavir/ritonavir or interferon-beta1b improves outcome of MERS-CoV infection in a nonhuman primate model of common marmoset. J. Infect. Dis. 212, 1904–1913.

Chen, Z., Bao, L., Chen, C., Zou, T., Xue, Y., Li, F., Lv, Q., Gu, S., Gao, X., Cui, S., Wang, J., Qin, C., Jin, Q., 2017. Human neutralizing monoclonal antibody inhibition of Middle East respiratory syndrome coronavirus replication in the common marmoset. J. Infect. Dis. 215, 1807–1815.

Corman, V.M., Eckerle, I., Bleicker, T., Zaki, A., Landt, O., Eschbach-Bludau, M., van Boheemen, S., Gopal, R., Ballhause, M., Bestebroer, T.M., Muth, D., Muller, M.A., Drexler, J.F., Zambon, M., Osterhaus, A.D., Fouchier, R.M., Drosten, C., 2012. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. bulletin europeen sur les maladies transmissibles = European communicable disease bulletin 17.

Corti, D., Bianchi, S., Vanzetta, F., Minola, A., Perez, L., Agatic, G., Guarino, B., Silacci, C., Marcandalli, J., Marsland, B.J., Piralla, A., Percivalle, E., Sallusto, F., Baldanti, F., Lanzavecchia, A., 2013. Cross-neutralization of four paramyxoviruses by a human monoclonal antibody. Nature 501, 439–443.

Corti, D., Passini, N., Lanzavecchia, A., Zambon, M., 2016. Rapid generation of a human monoclonal antibody to combat Middle East respiratory syndrome. J. Infect. Public Health 9, 231–235.

Corti, D., Zhao, J., Pedotti, M., Simonelli, L., Agnihothram, S., Fett, C., Fernandez-Rodriguez, B., Foglierini, M., Agatic, G., Vanzetta, F., Gopal, R., Langrish, C.J., Barrett, N.A., Sallusto, F., Baric, R.S., Varani, L., Zambon, M., Perlman, S., Lanzavecchia, A., 2015. Prophylactic and postexposure efficacy of a potent human monoclonal antibody against MERS coronavirus. In: Proceedings of the National Academy of Sciences of the United States of America 112. pp. 10473–10478.

de Wit, E., Feldmann, F., Okumura, A., Horne, E., Haddock, E., Saturday, G., Scott, D., Erlandson, K.J., Stahl, N., Lipsich, L., Kyratsous, C.A., Feldmann, H., 2018. Prophylactic and therapeutic efficacy of mAb treatment against MERS-CoV in common marmosets. Antivir. Res. 156, 64–71.

de Wit, E., Rasmussen, A.L., Falzarano, D., Bushmaker, T., Feldmann, F., Brining, D.L., Fischer, E.R., Martellaro, C., Okumura, A., Chang, J., Scott, D., Benecke, A.G., Katze, M.G., Feldmann, H., Munster, V.J., 2013. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. In: Proceedings of the National Academy of Sciences of the United States of America 110. pp. 16598–16603.

Falzarano, D., de Wit, E., Feldmann, F., Rasmussen, A.L., Okumura, A., Peng, X., Thomas, M.J., van Doremalen, N., Haddock, E., Nagy, L., LaCasse, R., Liu, T., Zhu, J., McLellan, J.S., Scott, D.P., Katze, M.G., Feldmann, H., Munster, V.J., 2014. Infection

with MERS-CoV causes lethal pneumonia in the common marmoset. PLoS Pathog. 10 e1004250.
Falzarano, D., de Wit, E., Rasmussen, A.L., Feldmann, F., Okumura, A., Scott, D.P., Brining, D., Bushmaker, T., Martellaro, C., Baseler, L., Benecke, A.G., Katze, M.G., Munster, V.J., Feldmann, H., 2013. Treatment with interferon-alpha2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. Nat. Med. 19, 1313–1317.
Johnson, R.F., Bagci, U., Keith, L., Tang, X., Mollura, D.J., Zeitlin, L., Qin, J., Huzella, L., Bartos, C.J., Bohorova, N., Bohorov, O., Goodman, C., Kim do, H., Paulty, M.H., Velasco, J., Whaley, K.J., Johnson, J.C., Pettitt, J., Ork, B.L., Solomon, J., Oberlander, N., Zhu, Q., Sun, J., Holbrook, M.R., Olinger, G.G., Baric, R.S., Hensley, L.E., Jahrling, P.B., Marasco, W.A., 2016. 3B11-N, a monoclonal antibody against MERS-CoV, reduces lung pathology in rhesus monkeys following intratracheal inoculation of MERS-CoV Jordan-n3/2012. Virology 490, 49–58.
Li, Y., Wan, Y., Liu, P., Zhao, J., Lu, G., Qi, J., Wang, Q., Lu, X., Wu, Y., Liu, W., Zhang, B., Yuen, K.Y., Perlman, S., Gao, G.F., Yan, J., 2015. A humanized neutralizing antibody against MERS-CoV targeting the receptor-binding domain of the spike protein. Cell Res. 25, 1237–1249.
Pascal, K.E., Coleman, C.M., Mujica, A.O., Kamat, V., Badithe, A., Fairhurst, J., Hunt, C., Strein, J., Berrebi, A., Sisk, J.M., Matthews, K.L., Babb, R., Chen, G., Lai, K.M., Huang, T.T., Olson, W., Yancopoulos, G.D., Stahl, N., Frieman, M.B., Kyratsous, C.A., 2015. Pre- and postexposure efficacy of fully human antibodies against Spike protein in a novel humanized mouse model of MERS-CoV infection. In: Proceedings of the National Academy of Sciences of the United States of America 112. pp. 8738–8743.
Qiu, H., Sun, S., Xiao, H., Feng, J., Guo, Y., Tai, W., Wang, Y., Du, L., Zhao, G., Zhou, Y., 2016. Single-dose treatment with a humanized neutralizing antibody affords full protection of a human transgenic mouse model from lethal Middle East respiratory syndrome (MERS)-coronavirus infection. Antivir. Res. 132, 141–148.
van Doremalen, N., Falzarano, D., Ying, T., de Wit, E., Bushmaker, T., Feldmann, F., Okumura, A., Wang, Y., Scott, D.P., Hanley, P.W., Feldmann, H., Dimitrov, D.S., Munster, V.J., 2017. Efficacy of antibody-based therapies against Middle East respiratory syndrome coronavirus (MERS-CoV) in common marmosets. Antivir. Res. 143, 30–37.
Zhao, G., He, L., Sun, S., Qiu, H., Tai, W., Chen, J., Li, J., Chen, Y., Guo, Y., Wang, Y., Shang, J., Ji, K., Fan, R., Du, E., Jiang, S., Li, F., Du, L., Zhou, Y., 2018. A novel nanobody targeting Middle East respiratory syndrome coronavirus (MERS-CoV) receptor-binding domain has potent cross-neutralizing activity and protective efficacy against MERS-CoV. J. Virol. 92.