

1 **High efficacy of therapeutic equine hyperimmune antibodies against SARS-CoV-2**  
2 **variants of concern**

3

4 Andres Moreira-Soto<sup>1,2</sup>, Mauricio Arguedas<sup>3</sup>, Hebleen Brenes<sup>4</sup>, Willem Buján<sup>5,6</sup>, Eugenia  
5 Corrales-Aguilar<sup>2</sup>, Cecilia Díaz<sup>3,5</sup>, Ann Echeverri<sup>6</sup>, Marietta Flores-Díaz<sup>3</sup>, Aarón Gómez<sup>3</sup>,  
6 Andrés Hernández<sup>3</sup>, María Herrera<sup>3</sup>, Guillermo León<sup>3</sup>, Román Macaya<sup>6</sup>, Arne Kühne<sup>1</sup>, José  
7 Arturo Molina-Mora<sup>2</sup>, Javier Mora<sup>2</sup>, Alfredo Sanabria<sup>6</sup>, Andrés Sánchez<sup>3</sup>, Laura Sánchez<sup>3</sup>,  
8 Álvaro Segura<sup>3</sup>, Eduardo Segura<sup>3</sup>, Daniela Solano<sup>3</sup>, Claudio Soto<sup>4</sup>, Jennifer L. Stynoski<sup>3</sup>,  
9 Mariángela Vargas<sup>3</sup>, Mauren Villalta<sup>3</sup>, Chantal B E M Reusken<sup>7</sup>, Christian Drosten<sup>1</sup>, José  
10 María Gutiérrez<sup>3</sup>, Alberto Alape-Girón<sup>3,5</sup>, Jan Felix Drexler<sup>1,8#</sup>

11

12 <sup>1</sup>Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin,  
13 Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Virology, 10117  
14 Berlin, Germany

15 <sup>2</sup>Centro de Investigación en Enfermedades Tropicales (CIET), Facultad de Microbiología,  
16 Universidad de Costa Rica, Costa Rica

17 <sup>3</sup>Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, Costa  
18 Rica

19 <sup>4</sup>Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud, Ministry of  
20 Health, Costa Rica

21 <sup>5</sup>School of Medicine, Universidad de Costa Rica, Costa Rica

22 <sup>6</sup>Caja Costarricense del Seguro Social, Costa Rica

23 <sup>7</sup>Centre for Infectious Disease Control, National Institute for Public Health and the  
24 Environment, Bilthoven, the Netherlands.

25 <sup>8</sup>German Centre for Infection Research (DZIF)

26 # Corresponding author

27

28 **Corresponding author contact information**

29 Professor Dr. Jan Felix Drexler, Helmut-Ruska-Haus, Institute of Virology, Campus Charité

30 Mitte, Charitéplatz 1, 10098 Berlin, Germany, Tel.: +49 30 450 525461/Fax: +49 30 450

31 525907, felix.drexler@charite.de

32

33 Word count, abstract: 50

34 Word count, text: 703

35

36 **Competing Financial Interests Statement**

37 The authors declare no competing financial interests.

38

39 **Abstract**

40 SARS-CoV-2 variants of concern (VoC) show reduced neutralization by vaccine-induced and  
41 therapeutic monoclonal antibodies. We tested therapeutic equine polyclonal antibodies  
42 (pAbs) against four VoC (alpha, beta, epsilon and gamma). We show that equine pAbs  
43 efficiently neutralize VoC, suggesting they are an effective, broad coverage, low-cost and a  
44 scalable COVID-19 treatment.

45

46 **To the editor:** SARS-CoV-2 causes coronavirus infectious disease 19 (COVID-19), which  
47 leads to either critical illness or death in 5% of patients (1). COVID-19 prevention and  
48 treatment options include vaccines, antivirals, and antibody formulations. A wide array of  
49 vaccine platforms have shown efficacy in preventing severe disease, but universal access is  
50 limited and many resource-limited settings largely lack sufficient vaccine coverage (2). Even  
51 though there are more than 300 therapeutic drugs in clinical trials, few have proven  
52 advantageous, such as dexamethasone (1, 3). Direct-acting antivirals like Remdesivir are  
53 most effective if given very early, require supplementary oxygen therapy and are very costly  
54 at 2,000-3,000 USD per treatment, limiting universal access (4). Convalescent plasma or  
55 hyperimmune globulins, which can be prepared from the pooling of many donors, have been  
56 used for decades to treat diseases such as ebola and influenza and could be a more affordable  
57 at 350-1,000 USD per treatment. However, their preparation is donor-dependent, requires  
58 strict donor rigorous testing for both blood-borne pathogens and high levels of neutralizing  
59 anti-SARS-CoV-2 antibodies, not readily available on blood bank systems in many  
60 developing countries (5). The use of monoclonal antibodies (mAbs) are safe alternatives  
61 shown to enhance viral clearance, but their large scale production is challenging and costly, at  
62 around 1,500-6,500 USD per treatment (6). A low-cost alternative to mAbs are formulations  
63 of intact or fragmented equine polyclonal antibodies (pAbs), widely used for decades as  
64 therapies against viral infections or as antivenoms.

65 We and others have previously shown that horses can be efficiently immunized with different  
66 SARS-CoV-2 antigens to yield high quantities of purified polyclonal antibodies (pAbs) that  
67 are 50-80 times more potent than convalescent plasma (7, 8). A formulation of equine  
68 polyclonal F(ab')<sub>2</sub> fragments against the receptor binding domain (RBD) of SARS-CoV-2  
69 was tested in a multi-center, double-blind, placebo-controlled phase II/III clinical trial  
70 showing that it is well tolerated and leads to clinical improvement of hospitalized patients

71 with moderate to severe COVID-19 (9). Additionally, there is an ongoing randomized, multi-  
72 center, double-blind, placebo-controlled, dose-finding, phase IIb/III clinical trial  
73 (NCT04838821) at hospitals of the Costa Rican Social Security Fund testing equine pAbs  
74 formulations to treat moderate and severe COVID-19 cases.

75 However, pre-clinical data of equine hyperimmune pAbs are only available for early SARS-  
76 CoV-2 isolates, such data are lacking for recent and globally circulating variants, considered  
77 of concern (VoC) due to their increased transmissibility. Voc alpha, beta, epsilon and gamma  
78 (<https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html>) (lineage  
79 designations in Pango/Nextrain: B.1.1.7/501Y.V1 first detected in the United Kingdom,  
80 B.1.351/501Y.V2 first detected in South Africa, P.1/501Y.V3 first detected in Brazil/Japan,  
81 and B.1.427/B.1.429 first detected in the US/California) exhibit a substantial reduction or  
82 complete abrogation of neutralization by therapeutic mAbs or by antibodies present in the  
83 plasma of vaccinated or convalescent individuals (10).

84 Here we report the results of a plaque reduction neutralization assay against VoC for our  
85 purified equine pAbs formulations. The two formulations are the SARS-CoV-2 recombinant  
86 S1 protein (called anti-S1; produced in baculovirus insect cells), and SEM mosaic (called  
87 anti-mix; an *E. coli* derived recombinant protein containing the S, E, and M immunodominant  
88 regions) derived from the strain Wuhan-Hu-1, Accession N YP\_009724390 (Native Antigen  
89 Company, Oxford, United Kingdom), purified using caprylic acid precipitation method (8).  
90 Both formulations effectively neutralized four VoC and an early isolate of the virus  
91 (Germany/Gisaid\_EPI\_ISL\_406862) at similar inhibitory concentrations (IC<sub>50</sub> range for anti-  
92 S1 formulation: 0.206-0.377 µg/mL; and for the anti-mix formulation: 0.146-0.471 µg/mL;  
93 **Figure 1**; IC<sub>50</sub> dose-response curves are shown in the Technical Annex). Those  
94 concentrations are extremely low when compared to pAbs doses used by other groups in

95 patients in clinical trials (4 mg/kg) (9), even at the upper estimates of the 95% confidence  
96 intervals, reaching a maximum of 13.89  $\mu\text{g/mL}$  for the beta (B.1.351/501Y.V2) VoC. For  
97 both equine pAbs formulations the differences between potencies against tested VoC and  
98 early SARS-CoV-2 isolates were not statistically significant (sum-of-squares F test of Anti-  
99 S1;  $p=0.9$ , Anti-Mix,  $p=0.8$ ).

100 Our data suggest high potential of equine pAbs for treatment of COVID-19. By shifting  
101 antivenom platforms to produce equine pAbs, laboratories in both developed and developing  
102 countries that have been manufacturing and distributing safe and standardized antivenoms for  
103 decades could rapidly fill the gaps in global demand for therapies that are both effective  
104 against VoC and affordable to low- and middle-income countries.

#### 105 **Declaration of interests**

106 We declare no competing interests.

#### 107 **Acknowledgements**

108 This work was supported by the “Global Centres for Health and Pandemic Prevention” from  
109 the German academic exchange services (DAAD).

110

#### 111 **References**

112

- 113 1. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat*  
114 *Rev Microbiol.* 2021;19(3):141-54.
- 115 2. Bollyky TJ, Gostin LO, Hamburg MA. The Equitable Distribution of COVID-19  
116 Therapeutics and Vaccines. *JAMA.* 2020;323(24):2462-3.
- 117 3. Dexamethasone in Hospitalized Patients with Covid-19. *New England Journal of*  
118 *Medicine.* 2020;384(8):693-704.

- 119 4. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al.  
120 Remdesivir for the Treatment of Covid-19 — Final Report. *New England Journal of*  
121 *Medicine*. 2020;383(19):1813-26.
- 122 5. The Lancet H. The resurgence of convalescent plasma therapy. *Lancet Haematol*.  
123 2020;7(5):e353-e.
- 124 6. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhoire R, et al. REGN-  
125 COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. *New England*  
126 *Journal of Medicine*. 2020;384(3):238-51.
- 127 7. Zylberman V, Sanguineti S, Pontoriero AV, Higa SV, Cerutti ML, Morrone Seijo SM,  
128 et al. Development of a hyperimmune equine serum therapy for COVID-19 in Argentina.  
129 *Medicina*. 2020;3:1-6.
- 130 8. León G, Herrera M, Vargas M, Arguedas M, Sánchez A, Segura Á, et al.  
131 Development and characterization of two equine formulations towards SARS-CoV-2 proteins  
132 for the potential treatment of COVID-19. *Scientific reports*. 2021;11(1):021-89242.
- 133 9. Lopardo G, Belloso WH, Nannini E, Colonna M, Sanguineti S, Zylberman V, et al.  
134 RBD-specific polyclonal F(ab')<sub>2</sub> fragments of equine antibodies in patients with moderate  
135 to severe COVID-19 disease: A randomized, multicenter, double-blind, placebo-controlled,  
136 adaptive phase 2/3 clinical trial. *EClinicalMedicine*. 2021;34(100843):11.
- 137 10. Chen RE, Zhang X, Case JB, Winkler ES, Liu Y, VanBlargan LA, et al. Resistance of  
138 SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal  
139 antibodies. *Nat Med*. 2021;27(4):717-26.

140  
141

142

143 **Figure 1.** *In vitro* neutralizing potency of (A) Anti-S1 (S1 SARS-CoV-2 recombinant  
144 protein) and (B) Anti-Mix (mixture of S1, N, and SEM mosaic SARS-CoV-2 recombinant  
145 proteins of Wuhan-Hu-1, Accession N YP\_009724390.1) polyclonal antibodies purified from  
146 the plasma of hyperimmunized horses against different SARS-CoV-2 variants of concern  
147 (VoC) and a early isolate, named using WHO and Pango/Nextrain designations (strains used=  
148 GERMANY/GISAID EPI\_ISL 406862, BetaCoV/ChVir21652, hCoV-  
149 19/Aruba\_11401/2021, hCoV-19/Netherlands/NoordHolland\_10915/2021,  
150 BetaCoV/ChVir22131/B.1.351/501Y.V2, acquired from <https://www.european-virus->  
151 [archive.com/evag-news/sars-cov-2-collection](https://www.european-virus-archive.com/evag-news/sars-cov-2-collection)). The inhibitory concentration (IC<sub>50</sub>) in plaque  
152 reduction neutralization tests (PRNT) was calculated using a nonlinear regression analysis in  
153 the GraphPadPrism 5 software. Potencies (IC<sub>50</sub>) were not statistically different among viral  
154 variants with either formulation, and the null hypothesis was not rejected, meaning the IC<sub>50</sub>  
155 was equal in all datasets. Dotted lines denote the mean minimum and maximum  
156 concentration and solid lines denote 95% confidence intervals for both formulations. Plaque  
157 reduction neutralization tests (PRNT) were performed as follows. Briefly, VeroE6 cells (3.25  
158 × 10<sup>5</sup> cells/ml) were seeded in 24-well plates and incubated overnight. Equine polyclonal  
159 antibody formulations were mixed in equal parts with a virus solution containing 20 PFU.

160 The serum–virus solution was incubated at 37°C for 1 h and added to the cells. After 1 h at  
161 37°C, supernatants were discarded, and cells were supplemented with 1.2% Avicel solution  
162 in DMEM. After 3 d at 37°C, supernatants were removed, and the 24-well plates were fixed  
163 and inactivated using a 6% formaldehyde/PBS solution and stained with crystal violet, and  
164 plaques were counted.

165

166

167

168 **Annex figure.** IC50 dose-response curves to SARS-CoV-2 early isolates and variants of  
169 concern named using WHO and Pango/Nextrain designations. The Y axis denotes the mean  
170 plaque forming units (PFU) per milliliter in triplicate. The X axis denotes the Log10  
171 concentration of the Anti-S1 and the Anti-Mix (combination of S1, N and SEM mosaic  
172 protein of Wuhan-Hu-1, Accession N YP\_009724390.1) formulations.

173

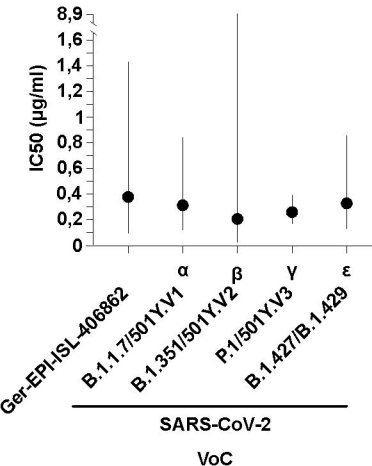
#### 174 **First author biographical sketch**

175 Dr. Moreira-Soto is a virologist at the Charité-Universitätsmedizin Berlin Institute of  
176 Virology and the University of Costa Rica Research Center for Tropical Diseases (CIET). His  
177 research interests include virology, epidemiology, public health, and molecular biology of  
178 emerging infectious diseases.



# A

## Anti-S1



**B** Anti-Mix (S1, N, and SEM)