

# Kent Academic Repository

## Full text document (pdf)

### Citation for published version

Bojkova, Denisa, Stack, Richard, Rothenburger, Tamara, Kandler, Joshua D., Ciesek, Sandra, Wass, Mark N., Michaelis, Martin and Cinatl, Jindrich (2022) Synergism of interferon-beta with antiviral drugs against SARS-CoV-2 variants. *Journal of Infection*, 85 (5). pp. 573-607. ISSN 0163-4453.

### DOI

<https://doi.org/10.1016/j.jinf.2022.07.023>

### Link to record in KAR

<https://kar.kent.ac.uk/97539/>

### Document Version

Author's Accepted Manuscript

#### Copyright & reuse

Content in the Kent Academic Repository is made available for research purposes. Unless otherwise stated all content is protected by copyright and in the absence of an open licence (eg Creative Commons), permissions for further reuse of content should be sought from the publisher, author or other copyright holder.

#### Versions of research

The version in the Kent Academic Repository may differ from the final published version.

Users are advised to check <http://kar.kent.ac.uk> for the status of the paper. **Users should always cite the published version of record.**

#### Enquiries

For any further enquiries regarding the licence status of this document, please contact:

[researchsupport@kent.ac.uk](mailto:researchsupport@kent.ac.uk)

If you believe this document infringes copyright then please contact the KAR admin team with the take-down information provided at <http://kar.kent.ac.uk/contact.html>

1 **Synergism of interferon-beta with antiviral drugs against SARS-CoV-2**  
2 **variants**

3 Denisa Bojkova<sup>1</sup>, Richard Stack<sup>2</sup>, Tamara Rothenburger<sup>1</sup>, Joshua D Kandler<sup>1</sup>, Sandra  
4 Ciesek<sup>1,3,4</sup>, Mark N. Wass<sup>2\*</sup>, Martin Michaelis<sup>2\*</sup>, Jindrich Cinatl jr.<sup>1,5\*</sup>

5 <sup>1</sup> Institute for Medical Virology, University Hospital, Goethe University, Frankfurt am  
6 Main, Germany

7 <sup>2</sup> School of Biosciences, University of Kent, Canterbury, UK

8 <sup>3</sup> German Center for Infection Research, DZIF, External partner site, Frankfurt am  
9 Main, Germany

10 <sup>4</sup> Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Branch  
11 Translational Medicine und Pharmacology, Frankfurt am Main, Germany

12 <sup>5</sup> Dr. Petra Joh-Forschungshaus, Frankfurt am Main, Germany

13

14 \* Corresponding authors:

15 Jindrich Cinatl jr., Institute for Medical Virology, University Hospital, Goethe University,  
16 Paul Ehrlich-Straße 40, 60596 Frankfurt am Main, Germany; phone +49 69 6301  
17 6409; e-mail [Cinatl@em.uni-frankfurt.de](mailto:Cinatl@em.uni-frankfurt.de)

18 Martin Michaelis, School of Biosciences, University of Kent, Canterbury CT2 7NJ, UK;  
19 phone +44 1227 82 7804; e-mail [M.Michaelis@kent.ac.uk](mailto:M.Michaelis@kent.ac.uk)

20 Mark N. Wass, School of Biosciences, University of Kent, Canterbury CT2 7NJ, UK;  
21 phone +44 1227 82 7626; e-mail [M.N.Wass@kent.ac.uk](mailto:M.N.Wass@kent.ac.uk)

22

23 **Keywords:** SARS-CoV-2; COVID-19; antiviral therapy; interferon; combination  
24 therapy; nirmatrelvir; molnupiravir; remdesivir; aprotinin

25

26 To the Editor,

27 In their recent article, Vellas et al. reported that tixagevimab-cilgavimab  
28 treatment of COVID-19 patients induces resistance mutations in SARS-CoV-2  
29 Omicron BA.2 [Vellas et al., 2022], contributing to concerns that resistance formation  
30 may affect the efficacy of anti-SARS-CoV-2 therapies. In this context, more effective  
31 combination therapies are anticipated to reduce resistance formation [White et al.,  
32 2021].

33 Interferons are potential anti-SARS-CoV-2 drugs but displayed limited efficacy  
34 in initial clinical trials for the treatment of COVID-19 [WHO Solidarity Trial Consortium,  
35 2021]. Based on findings that Omicron variant BA.1 isolates replicated less effectively  
36 in interferon-competent cells and were more sensitive to interferon treatment than a  
37 Delta isolate [Bojkova et al., 2022; Bojkova et al., 2022a], we here systematically  
38 compared the sensitivity of Delta, BA.1, and BA.2 isolates to betaferon (a clinically  
39 approved interferon- $\beta$  preparation) alone or in combination with the approved anti-  
40 SARS-CoV-2 drugs remdesivir (RNA-dependent RNA polymerase inhibitor), EIDD-  
41 1931 (the active metabolite of molnupiravir that induces 'lethal mutagenesis' during  
42 virus replication), nirmatrelvir (inhibitor of the SARS-CoV-2 main/ 3CL protease, the  
43 antivirally active agent in Paxlovid), and aprotinin, a protease inhibitor that inhibits  
44 SARS-CoV-2 replication [Bojkova et al., 2020] and that was recently reported to be  
45 effective in COVID-19 patients in a clinical trial [Redondo-Calvo et al., 2022].

46 A comparison of sequence variants in Delta, Omicron BA.1, and Omicron BA.2  
47 virus isolates identified 96 sequence variants in putative viral interferon antagonists  
48 that differed from the reference genome of the original Wuhan strain (Suppl. Table 1).  
49 The overlap in sequence variants between BA.1 and BA.2 was larger (49) than  
50 between Delta and BA.1 (21) and Delta and BA.2 (18). Moreover, Delta displayed

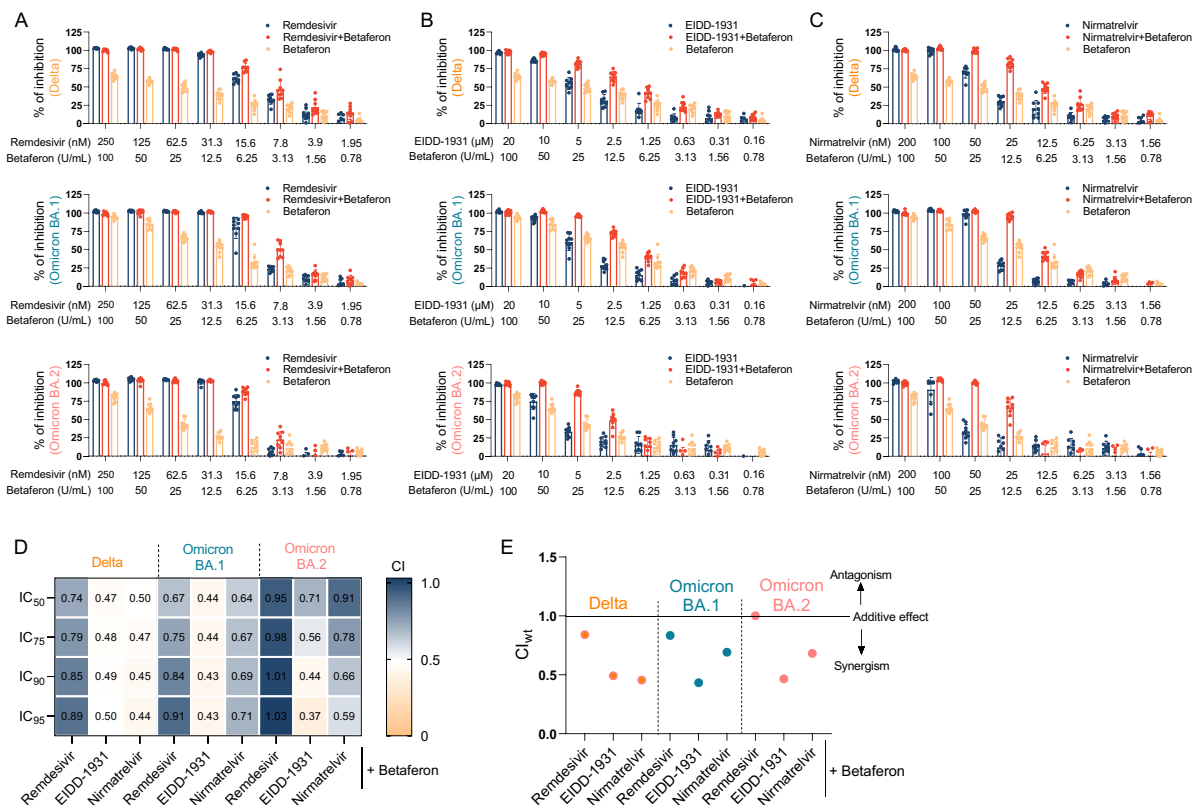
51 more unique sequence variants (54) than BA.1 (23) or BA.2 (26) (Suppl. Figure 1A).  
52 These findings appear to reflect the closer relatedness of BA.1 and BA.2 relative to  
53 Delta. However, the variant overlaps are complex (Suppl. Figure 1B, Suppl. File 1),  
54 and it is not clear, which of them drive the virus response to interferons. Of the 45 of  
55 the 96 sequence variants that could be modelled on protein structures or models  
56 (Suppl. File 1), only two were proposed to have a likely impact on interferon signalling  
57 based on an *in silico* structural analysis (Suppl. Figure 1, Suppl. Table 1, Suppl. File  
58 1). These findings warrant the further comparison of Delta, BA.1, and BA.2 variants  
59 for their responses to interferon treatment. Indeed, a BA.2 isolate replicated more  
60 effectively than BA.1 but less effectively than Delta in Caco-2-F03 cells, a Caco-2  
61 subline that is highly susceptible to SARS-CoV-2 infection [Bojkova et al., 2022b]  
62 (Suppl. Figure 2).

63         Next, we tested the effects of remdesivir, EIDD-1931, and nirmatrelvir on Delta,  
64 BA.1, and BA.2 replication. Delta and BA.1 displayed similar sensitivity to the  
65 approved anti-SARS-CoV-2 drugs remdesivir, nirmatrelvir, and EIDD-1931, whereas  
66 BA.2 was less sensitive to EIDD-1931 than Delta and BA.1 (Suppl. Figure 3).

67         In agreement with previous findings [Bojkova et al., 2022] the clinically  
68 approved interferon- $\beta$  preparation betaferon (Bayer) was more effective against BA.1  
69 than against Delta (Suppl. Figure 3). Interestingly and perhaps unexpectedly, the  
70 betaferon response of BA.2 more closely resembled that of Delta and not that of the  
71 more closely related BA.1 (Suppl. Figure 3). This confirmed our previous findings  
72 (Suppl. Figure 1) that the impact of amino acid sequence differences in different  
73 SARS-CoV-2 isolates on the viral interferon response is not easily predictable and can  
74 differ even between closely related virus variants.

75 Among the tested antiviral drugs, remdesivir was the only one that did not  
 76 display synergistic effects in combination with betaferon (Figure 1), which may reflect  
 77 clinical findings indicating that the addition of interferon does not increase remdesivir  
 78 efficacy in COVID-19 patients [Kalil et al., 2021]. While EIDD-1931 and nirmatrelvir  
 79 treatment resulted in similar levels of synergism with betaferon against Delta,  
 80 combined EIDD-1931 and interferon treatment was associated with a more  
 81 pronounced synergism against BA.1 and BA.2 than the combination of nirmatrelvir  
 82 and betaferon (Figure 1).  
 83

Figure 1



84  
 85 **Figure 1. Antiviral effects of approved anti-SARS-CoV-2 drugs in combination**  
 86 **with interferon-β (betaferon) against Delta, Omicron BA.1, and Omicron BA.2**  
 87 **isolates. Betaferon was tested in fixed combinations combination with remdesivir (A),**

88 EIDD-1931 (B), or nirmatrelvir (C) in SARS-CoV-2 (MOI 0.01)-infected Caco-2-F03  
89 cells. Values represent mean  $\pm$  S.D. of three independent experiments. D)  
90 Combination indices were calculated at the IC<sub>50</sub>, IC<sub>75</sub>, IC<sub>90</sub>, and IC<sub>95</sub> levels following  
91 the method of Chou and Talalay. E) The weighted average CI value (CI<sub>wt</sub>) was  
92 calculated according to the formula: CI<sub>wt</sub> [CI<sub>50</sub> + 2CI<sub>75</sub> + 3CI<sub>90</sub> + 4CI<sub>95</sub>]/10. A CI<sub>wt</sub> <1  
93 indicates synergism, a CI<sub>wt</sub> =1 indicates additive effects, and a CI<sub>wt</sub> >1 suggest  
94 antagonism.

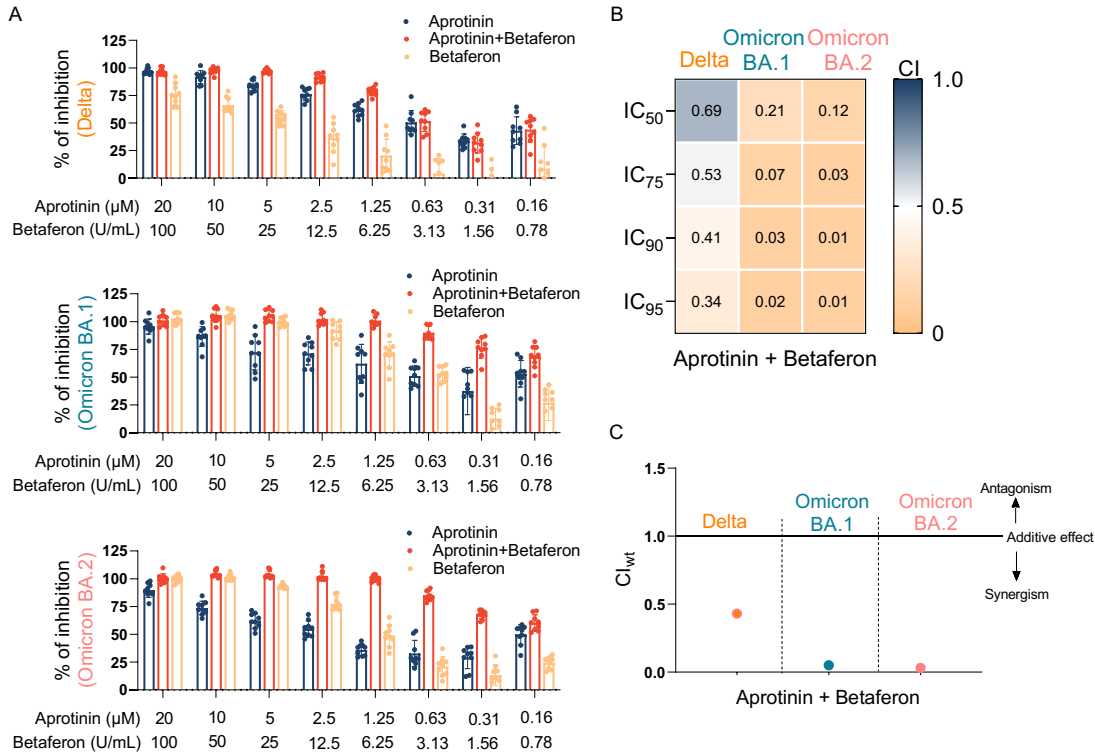
95

96 Aprotinin inhibited Delta (IC<sub>50</sub>: 0.66 $\mu$ M) and BA.1 (IC<sub>50</sub>: 0.64 $\mu$ M) in a similar  
97 concentration range as the original Wuhan strain isolates [Bojkova et al., 2020] (Suppl.  
98 Figure 4). Effects against BA.2 were less pronounced (IC<sub>50</sub>: 1.95 $\mu$ M) but still in the  
99 range of clinically achievable plasma concentrations after systemic administration,  
100 which have been shown to reach 11.8 $\mu$ M [Levy et al., 1994]. Moreover, aerosol  
101 preparations like the one used in the clinical trial that demonstrated therapeutic  
102 efficacy of aprotinin against COVID-19 [Redondo-Calvo et al., 2022] are expected to  
103 result in substantially higher local aprotinin concentrations in the lungs.

104 Aprotinin displayed the strongest synergism with betaferon against BA.1 and  
105 BA.2 among all tested drugs. Against Delta, the level of synergism of aprotinin/  
106 betaferon was similar to that of EIDD-1931/ betaferon (Figure 2).

107

Figure 2



108

109 **Figure 2. Antiviral effects of aprotinin in combination with interferon-β**

110 **(betaferon) against Delta, Omicron BA.1, and Omicron BA.2 isolates.** Betaferon

111 was tested in a fixed combination with aprotinin in SARS-CoV-2 (MOI 0.01)-infected

112 Caco-2-F03 cells. Values represent mean ± S.D. of three independent experiments.

113 B) Combination indices were calculated at the IC<sub>50</sub>, IC<sub>75</sub>, IC<sub>90</sub>, and IC<sub>95</sub> levels following

114 the method of Chou and Talalay. C) The weighted average CI value ( $CI_{wt}$ ) was

115 calculated according to the formula:  $CI_{wt} [CI_{50} + 2CI_{75} + 3CI_{90} + 4CI_{95}]/10$ . A  $CI_{wt} < 1$

116 indicates synergism, a  $CI_{wt} = 1$  indicates additive effects, and a  $CI_{wt} > 1$  suggest

117 antagonism.

118

119 In conclusion, even closely related SARS-CoV-2 (sub)variants can differ in their

120 biology, as indicated by different BA.1 and BA.2 replication kinetics, and in their

121 response to antiviral treatments, as indicated by differences in the virus responses to

122 betaferon, EIDD-1931/ molnupiravir, and aprotinin and differing levels of synergism of

123 betaferon combinations with other antiviral drugs. Betaferon combinations with  
124 nirmatrelvir and, in particular, with EIDD-1931 and aprotinin displayed high levels of  
125 synergism, which makes them strong candidates for clinical testing.

126



127 **Acknowledgements**

128           We thank Lena Stegman, Kerstin Euler, and Sebastian Grothe for their  
129 technical assistance.

130 **Funding**

131           This work was supported by the Frankfurter Stiftung für krebskranke Kinder,  
132 the Goethe-Corona-Fonds, the Corona Accelerated R&D in Europe (CARE) project  
133 from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant  
134 agreement No 101005077, and the SoCoBio DTP (BBSRC).

135 **Competing interests**

136           The authors declare no competing interests.

137

138

139 **References**

- 140 Bojkova D, Bechtel M, McLaughlin KM, McGreig JE, Klann K, Bellinghausen C, Rohde  
141 G, Jonigk D, Braubach P, Ciesek S, Münch C, Wass MN, Michaelis M, Cinatl J Jr.  
142 Aprotinin inhibits SARS-CoV-2 replication. *Cells* 2020;9:2377.
- 143 Bojkova D, Rothenburger T, Ciesek S, Wass MN, Michaelis M, Cinatl J Jr. SARS-CoV-  
144 2 Omicron variant virus isolates are highly sensitive to interferon treatment. *Cell*  
145 *Discov.* 2022 May 10;8(1):42.
- 146 Bojkova D, Widera M, Ciesek S, Wass MN, Michaelis M, Cinatl J jr. Reduced interferon  
147 antagonism but similar drug sensitivity in Omicron variant compared to Delta variant  
148 SARS-CoV-2 isolates. *Cell Res.* 2022a Mar;32(3):319-321.
- 149 Bojkova D, Reus P, Panosch L, Bechtel M, Rothenburger T, Kandler J, Pfeiffer A,  
150 Wagner JUG, Shumliakivska M, Dimmeler S, Olmer R, Martin U, Vondran F, Toptan  
151 T, Rothweiler F, Zehner R, Rabenau H, Osman KL, Pullan ST, Carroll M, Stack R,  
152 Ciesek R, Wass MN, Michaelis M, Cinatl J Jr. Identification of novel antiviral drug  
153 candidates using an optimized SARS-CoV-2 phenotypic screening platform. *bioRxiv.*  
154 2022b Jul 17:2022.07.17.500346. doi: 10.1101/2022.07.17.500346.
- 155 Kalil AC, Mehta AK, Patterson TF, Erdmann N, Gomez CA, Jain MK, Wolfe CR, Ruiz-  
156 Palacios GM, Kline S, Regalado Pineda J, Luetkemeyer AF, Harkins MS, Jackson  
157 PEH, Iovine NM, Tapson VF, Oh MD, Whitaker JA, Mularski RA, Paules CI, Ince D,  
158 Takasaki J, Sweeney DA, Sandkovsky U, Wyles DL, Hohmann E, Grimes KA,  
159 Grossberg R, Laguio-Vila M, Lambert AA, Lopez de Castilla D, Kim E, Larson L, Wan  
160 CR, Traenkner JJ, Ponce PO, Patterson JE, Goepfert PA, Sofarelli TA, Mocherla S,  
161 Ko ER, Ponce de Leon A, Doernberg SB, Atmar RL, Maves RC, Dangond F, Ferreira  
162 J, Green M, Makowski M, Bonnett T, Beresnev T, Ghazaryan V, Dempsey W, Nayak  
163 SU, Dodd L, Tomashek KM, Beigel JH; ACTT-3 study group members. Efficacy of

164 interferon beta-1a plus remdesivir compared with remdesivir alone in hospitalised  
165 adults with COVID-19: a double-blind, randomised, placebo-controlled, phase 3 trial.  
166 *Lancet Respir Med.* 2021 Dec;9(12):1365-1376. doi: 10.1016/S2213-2600(21)00384-  
167 2.

168 Levy JH, Bailey JM, Salmenperä M. Pharmacokinetics of aprotinin in preoperative  
169 cardiac surgical patients. *Anesthesiology.* 1994 May;80(5):1013-8. doi:  
170 10.1097/00000542-199405000-00010.

171 Redondo-Calvo FJ, Padín JF, Muñoz-Rodríguez JR, Serrano-Oviedo L, López-Juárez  
172 P, Porrás Leal ML, González Gasca FJ, Rodríguez Martínez M, Pérez Serrano R,  
173 Sánchez Cadena A, Bejarano-Ramírez N, Muñoz Hornero C, Barberá Farré JR,  
174 Domínguez-Quesada I, Sepúlveda Berrocal MA, Villegas Fernández-Infantes MD,  
175 Manrique Romo MI, Parra Comino Á, Pérez-Ortiz JM, Gómez-Romero FJ; ATAC  
176 team. Aprotinin treatment against SARS-CoV-2: A randomized phase III study to  
177 evaluate the safety and efficacy of a pan-protease inhibitor for moderate COVID-19.  
178 *Eur J Clin Invest.* 2022 Jun;52(6):e13776.

179 Vellas C, Kamar N, Izopet J. Resistance mutations in SARS-CoV-2 omicron variant  
180 after tixagevimab-cilgavimab treatment. *J Infect.* 2022 Jul 22:S0163-4453(22)00422-  
181 4. doi: 10.1016/j.jinf.2022.07.014.

182 White JM, Schiffer JT, Bender Ignacio RA, Xu S, Kainov D, Ianevski A, Aittokallio T,  
183 Frieman M, Olinger GG, Polyak SJ. Drug Combinations as a First Line of Defense  
184 against Coronaviruses and Other Emerging Viruses. *mBio.* 2021 Dec  
185 21;12(6):e0334721. doi: 10.1128/mbio.03347-21.

186 WHO Solidarity Trial Consortium, Pan H, Peto R, Henao-Restrepo AM, Preziosi MP,  
187 Sathiyamoorthy V, Abdool Karim Q, Alejandria MM, Hernández García C, Kieny MP,  
188 Malekzadeh R, Murthy S, Reddy KS, Roses Periago M, Abi Hanna P, Ader F, Al-Bader

189 AM, Alhasawi A, Allum E, Alotaibi A, Alvarez-Moreno CA, Appadoo S, Asiri A, Aukrust  
190 P, Barratt-Due A, Bellani S, Branca M, Cappel-Porter HBC, Cerrato N, Chow TS,  
191 Como N, Eustace J, García PJ, Godbole S, Gotuzzo E, Giskevicius L, Hamra R,  
192 Hassan M, Hassany M, Hutton D, Irmansyah I, Jancoriene L, Kirwan J, Kumar S,  
193 Lennon P, Lopardo G, Lydon P, Magrini N, Maguire T, Manevska S, Manuel O,  
194 McGinty S, Medina MT, Mesa Rubio ML, Miranda-Montoya MC, Nel J, Nunes EP,  
195 Perola M, Portolés A, Rasmin MR, Raza A, Rees H, Reges PPS, Rogers CA, Salami  
196 K, Salvadori MI, Sinani N, Sterne JAC, Stevanovikj M, Tacconelli E, Tikkinen KAO,  
197 Trelle S, Zaid H, Røttingen JA, Swaminathan S. Repurposed Antiviral Drugs for Covid-  
198 19 - Interim WHO Solidarity Trial Results. *N Engl J Med.* 2021 Feb 11;384(6):497-511.  
199 doi: 10.1056/NEJMoa2023184.

200

201

202