Supplementary Appendix

Supplement to: Reis G, Silva EASM, Silva DCM, et al. Effect of early treatment with ivermectin among patients with Covid-19. N Engl J Med. DOI: 10.1056/NEJMoa2115869

This appendix has been provided by the authors to give readers additional information about the work.

(PDF updated April 5, 2022)

Trial Infrastructure and the Extended TOGETHER investigators

The COVID-19 TOGETHER Trial initiative was designed to evaluate repurposed treatments for COVID-19 disease through an adaptive trial design in two arms being conducted in Brazil.The trial is supported by a network of primary care research centers located in the state of Minas Gerais, Brazil, devoted to a comprehensive evaluation and treatment of patients with COVID-19. The trial was fully integrated with local public health authorities (Brazilian Unified Health System – SUS) as part of a coping strategy for the COVID-19 pandemic. Namely, the main institutions involved were: Cardresearch – Cardiologia Assistencial e de Pesquisa. This initiative is funded by FastGrants and The Rainwater Foundation.

The TOGETHER Trial consortium is a partnership between academics and clinicians at McMaster University in Ontario, Canada, and Pontifical Catholic University of Minas Gerais, Claros State University, and the Federal University of Ouro Preto in Minas Gerais, Brazil. Other partners include Cytel, Platform Life Sciences, MMS Holdings, WHO Therapeutic Guidelines Committee, and the Society for Clinical Trials.

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Clinical Events Classification Committee
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City of Sete Lagoas
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D'Oliveira Alves, Isadora Luiza Teixeira Neto, Jordanna Porto Inácio, Lara Silveira Marques, Larissa Martins do Nascimento, Larissa Oliveira Moreira, Lorena Luiza Soares de Oliveira, Lucas Eduardo Lessa Mussi, Lucas Medeiros Ruas, Luiza Nogueira Assis Barbosa, Marcelo Rodrigues da Costa Fernandes, Maria Eduarda Guimarães de Souza, Maria Thereza Figueiredo Bélem Calazans, Mariana Rodrigues Marinho de Bastos, Pedro Henrique Arcanjo Alvarenga, Quimberli Vassinave Cujuí, Rita de Cássia Silva, Vinícius Alves Corrêa, Vinícius Carvalho de Oliveira, Vitória Aparecida Cunha, Viviane Costa Santos

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Ana Beatriz Amorim, Carolina Francisca Martins, Eduardo Diniz Callegari, Huisnei Ferreira Lourenço, Jussara Carolina Assis Ribeiro Nascimento, Solange Santos Moreira Sales

City of Governador Valadares

Adhemar Dias de Figueiredo Neto, Adriana Marcos Mendes Rabelo, Aline do Carmo Rosa, Ana Paula Vilas Boas Wherberth, André Luiz Souza Gomes, Arthur Henrique Nunes Leite Oliveira, André Gustavo Pinto de Souza, Damiana Rogai Siqueira, Emanuel Peixoto Pinto, Felipe Coelho Soares de Oliveira, Felipe Fraga Damaceno, Guilherme Rhis, Iandra Silva Almeida, Igor Barros de Araújo Borges, Igor Brandão Rocha, Jéssica Genoveva Boline Passarelli Capaz Pinto da Silva, Jorge Carlos do Amaral Júnior, José Pio Marques II, Laura Lima Vargas, Lorena Cristina Ferreira Batista, Luiz Eduardo Coelho Fava, Luiza de Azevedo Freitas Giles, Marina Lacerda Marques, Maura Alina Morais Veríssimo, Neila Rodrigues Vargas de Paula, Pâmela de Sousa Dias Demuner, Ramail Santos Pouzas, Rafaella Rosa de Oliveira Fernandes, Roberta Coelho de Marco, Ronan Figueiredo Mourão, Sabrina Stefany da Silva Souza, Sara Silva Ferraz

Federal University of Ouro Preto, City of Ouro Preto
Aline Dias Bedetti, Anna Carolina Motta Costa, Cristiana Silva Mascarenhas, Diana Luiza Marinho Brandão, Felipe César Soares, Flávia Marcella Sena Gonçalves Borba, Karinna Guimarães Gomes, Leonardo Cançado Monteiro Savassi, Marina Eduarda Santos, Maryane de Oliveira Silva, Rayane Elen Fernandes Silva, Ruan Carling Schott Wondollinger, Yuri Barbosa de Menezes

Santa Casa de Misericórdia, City of Sabará
Bárbara Ellen Souza Rezende, Graziela César de Sousa, Graziele Aparecida Silva, Isabela Cristina de Oliveira Campos, Lara Silva Souza, Laura Nacife Rabello, Mariana Cristina Flores Emiliano, Roberta Ellen Santos Oliveira

Santa Rita Hospital, City of Contagem
Anna Clara de Jesus Oliveira, Bárbara Nogueira Roberti, Bruno Reis Garcia, Carina Daniela Lira Moreira Figueiredo, Eduardo Augusto dos Santos Moreira Silva, Júlia Diniz Assis Moreira, Heloisa Alves Costa, João Victor Barbosa Silveira, Mariana Campos Siqueira , Túlio Fonseca e Silva Quadros

Public health authorities and mayors
We are in debt with the following local public health authorities and mayors (listed by enrollment):

City of Ibirité
William Parreira Duarte (Mayor), Carina Bitarães (public health authority)

City of Sete Lagoas
Duílio de Castro (Mayor), Flávio Pimenta Silveira (public health authority), Alber Alípio Ribeiro (public health authority)

City of Betim
Vittorio Medioli (Mayor), Augusto Viana da Rocha (public health authority), Hilton Soares de Oliveira, Tânia Maria de Resende Amaral (public health authority)

City of Santa Luzia

Christiano Augusto Xavier Pereira (Mayor) Nádia Cristina Dias Duarte Tomé (public health authority)

City of Nova Lima
João Marcelo Diegues Pereira (Mayor), Diogo Jonata Ribeiro (public health authority)

City of Montes Claros
Humberto Guimarães Souto (Mayor), Dulce Pimenta Gonçalves (public health authority)

City of Brumadinho
Avimar de Melo Barcelos (Mayor), Eduardo Diniz Callegari (public health authority)

City of Governador Valadares
André Luiz Coelho Merlo (Mayor), Edna Gomes Leite, Caroline Martins Sangali (public health authority)

City of Ouro Preto
Angelo Oswaldo de Araujo Santos (Mayor), Glauciane Resende do Nascimento (public health authority)

Supplementary Tables and Figures

Supplemental Methods:

Trial Registration

This trial is a platform trial, and therefore, arms are added or dropped according to emerging data. This posology (400 mcg/kg) of the ivermectin evaluation versus placebo received Brazilian national ethics committee-CONEP approval on March 21, 2021, and the first patient enrolled on this posology was March 23, 2021. This evaluation of the trial was registered on clinicaltrials.gov on March 21, 2021. No patients included in this analysis were recruited prior to this date.

Description of Prolonged ER Visits and Modification of Primary Endpoint

When we initially proposed the study, we defined one of the endpoints as emergency care extended treatment of at least 12 hours. However, during the initial weeks of the beginning of the trial, we found that patients rarely stayed for more than 12 hours at emergency units for extended care and were later discharged home due to the progressive overcrowding of emergency units and referral centers for COVID-19. From March 2021, the health units in Minas Gerais State in Brazil experienced a depletion of their hospital bed capacities with >90% occupancy. During the period from May to mid-July, there was >100% occupancy of available hospital beds, leading to situations of "hospitalization" in the corridors of the units as there were no longer available hospital beds.

The lack of available hospitals to accommodate patients with moderate to severe COVID was then reflected in the emergency units, where the only option available to frontline medical teams was to release patients as quickly as possible to give others the opportunity to be treated with a minimum decent standard of medical care.

Thus, patients presenting with O_2 saturation between 85-93% and dyspnea without overt respiratory failure (i.e. FDA criteria of severe COVID-19)[1] were treated, undergoing initial respiratory stabilization, which included high-dose intravenous corticosteroids, supplemental oxygen, full inhalation therapy, and sometimes antibiotics, and a short stay at ER observation bed unit to monitor O_2 saturation and assess for progressive deterioration of respiratory status. Usually, after 4-6 hours, these patients under ER observation were re-evaluated with a decision made for being discharged home or hospitalized. In general, many ER patients are discharged home in less than 6 hours, and the majority of patients are discharged less than 12 hours so long as they are able to maintain their O_2 saturation at $\geq$90%. Patients discharged after prolonged ER observation were followed at a homecare program designed especially for persons with COVID-19. Persons unable to maintain their O_2 saturations above 90% were prioritized for hospital admission.

Rationale for modification of primary endpoint: Due to the limitations in health system capacity, we realized that a minimum observation period of 12 hours was unrealistic to capture participants with moderate/severe COVID-19. For this reason, we asked the National Research Ethics Commission in Brazil to modify the protocol endpoint to be 6 hours of ER observation instead of 12 hours. This change, based on the real world of care provided by the public emergency services of the Health System, was approved. This change was registered on clinicaltrials.gov on March 21, 2021. No data were analyzed prior to this change, and all blinding was maintained.

These persons presenting with O_2 saturation between 85-93% and dyspnea who underwent >6 hours of observation are consistent with the U.S. FDA definition of severe COVID-19.[1]

- Symptoms suggestive of severe systemic illness with COVID-19, which could include any symptom of moderate illness or shortness of breath at rest, or respiratory distress

- Clinical signs indicative of severe systemic illness with COVID-19, such as respiratory rate ≥ 30 per minute, heart rate ≥ 125 per minute, $SpO_2 \leq 93\%$ on room air at sea level or $PaO_2/FiO_2 < 300$

Description of Statistical Methods

All analyses involving dichotomous outcomes, including the primary outcome, were performed using the Bayesian beta-binomial model with uniform prior distributions for the individual arm event rates. Relative risks and posterior efficacy were evaluated based on size 10^6 Monte Carlo samples from the resultant Beta posterior distributions. The choice of uniform priors was, in part, made to minimize the impact of prior information or lack thereof, on the statistical inference. However, given the study size, no major impact of said choice was expected on the estimation, while interim analysis decision boundaries were calibrated to meet frequentist criteria of power and type I error rate. See the statistical analysis plan for more detail.

Time-to-event analyses that were not adjusted for competing risks, and numeric secondary outcomes, were performed using the default Bayesian implementation of the Cox proportional hazards model in the *brms* R library[2] with four independent Markov Chain Monte Carlo (MCMC) chains of size 4,000 each and a flat prior distribution assigned to the treatment assignment coefficient. For numeric outcomes, a Box-Cox power transformation was also applied to satisfy normality requirements. Finally, cause-specific Bayesian competing risks time-to-recovery analysis, adjusted for death, was performed using the method of Mahani and Sharabiani[3] with parametric Weibull models for the individual survival curves and MCMC samples of size 5,000. The 14-day restricted mean survival time difference[4] between the treatment groups was used as the treatment effect for reporting. Figures S1-S3 are purely descriptive and do not inform formal statistical inference.

Figure S1: Time to hospitalization or extended emergency room visit due to COVID-19

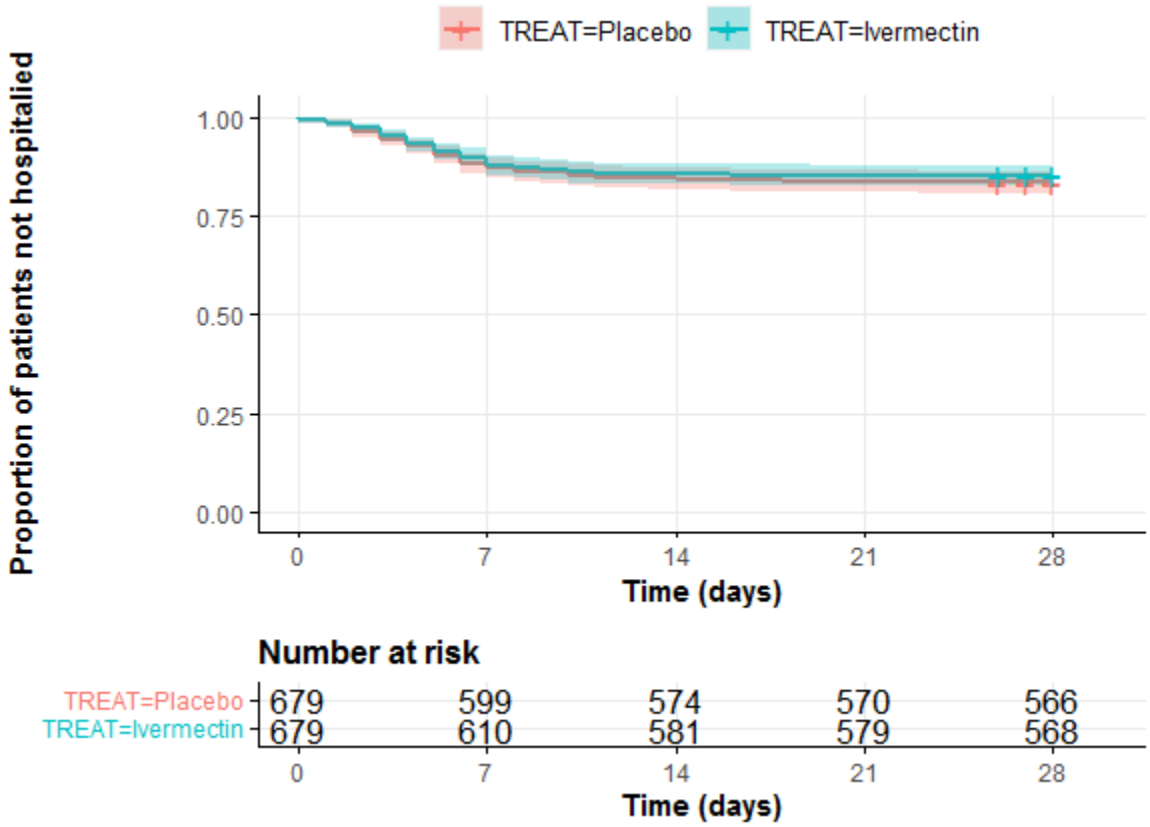


Figure S2: Time to clinical recovery (WHO clinical progression scale)

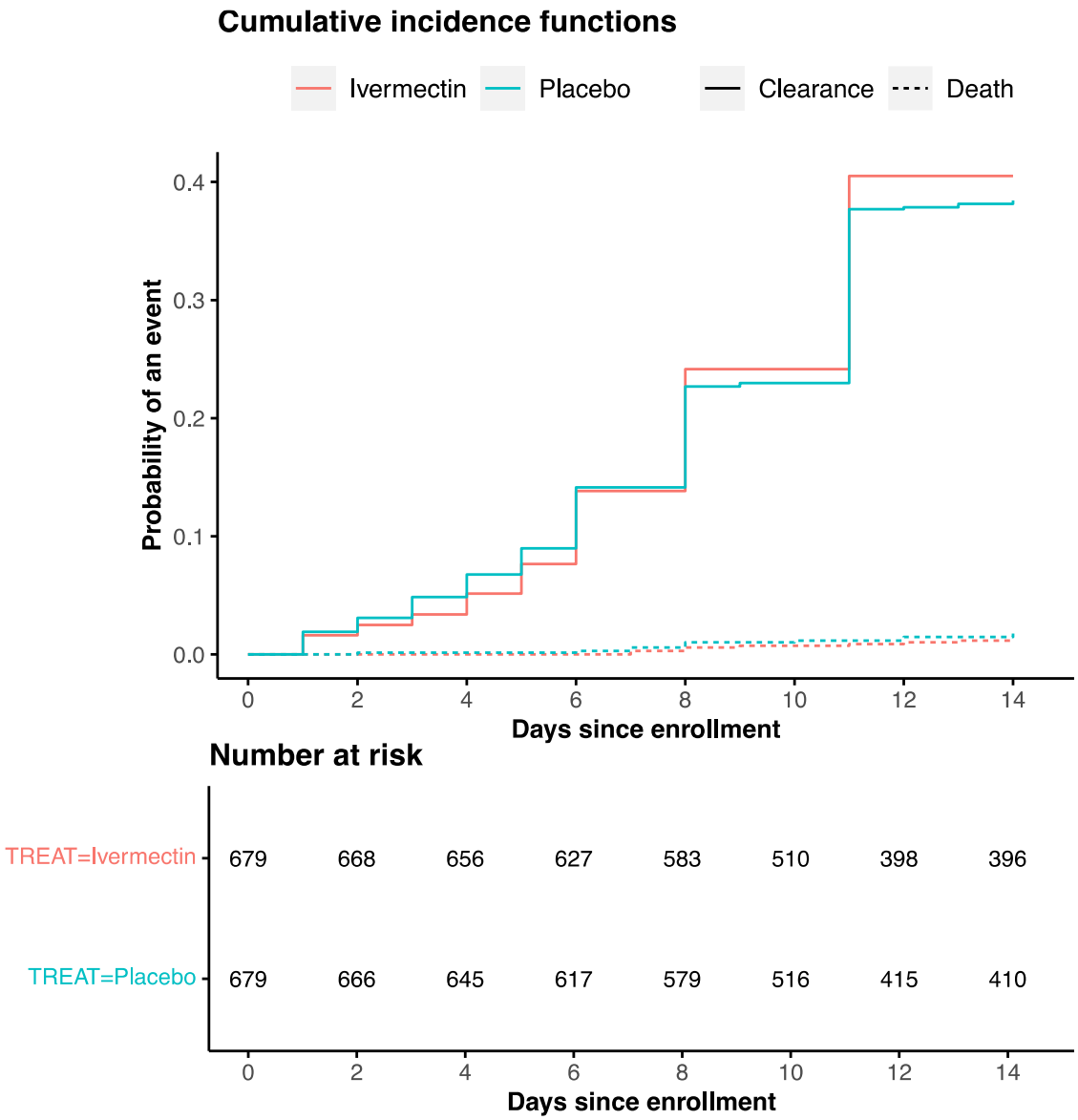
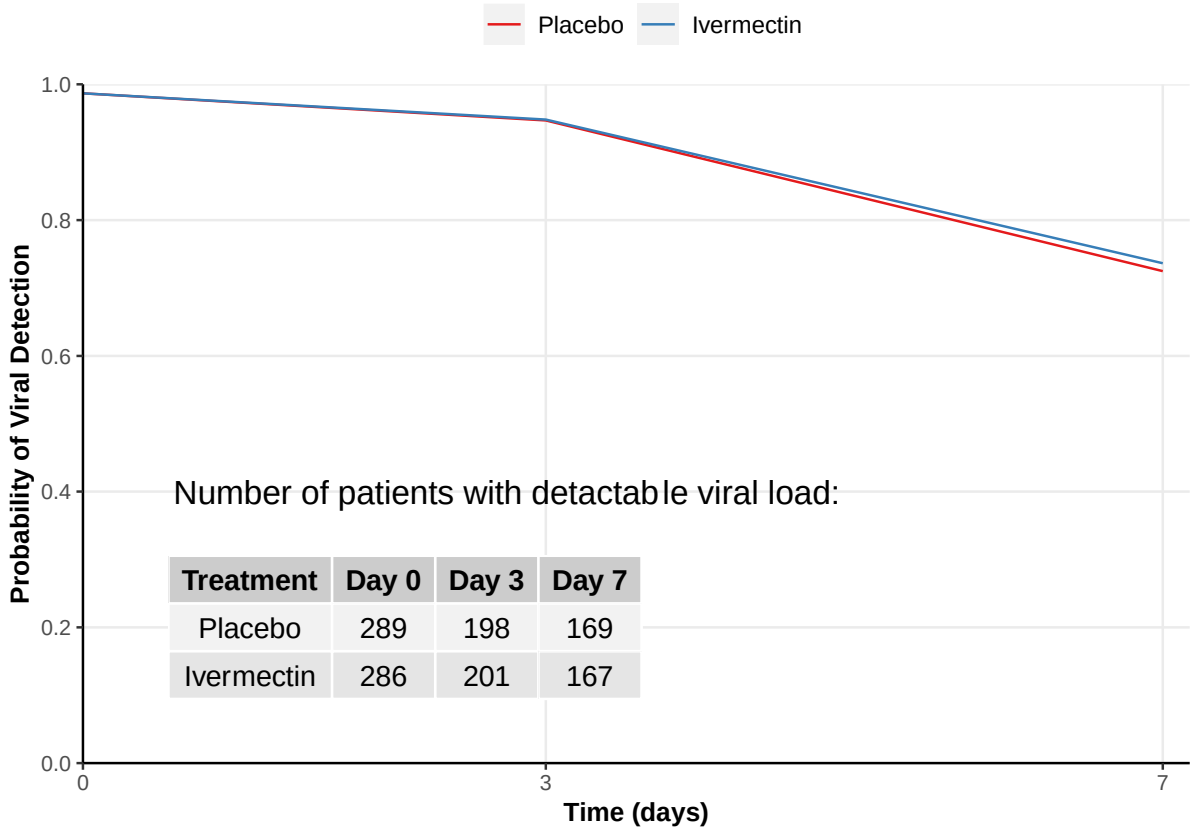


Figure S3: Probability of viral detection (Days 0, 3, 7)



Treatment	Day 0	Day 3	Day 7
Placebo	289	198	169
Ivermectin	286	201	167

Figure S4. PBPK model simulated (lines; mean and 5th and 95th percentiles) and observed (symbols) plasma concentration-time profiles for ivermectin after 30 mg oral doses administered on Days 1, 4 and 7.

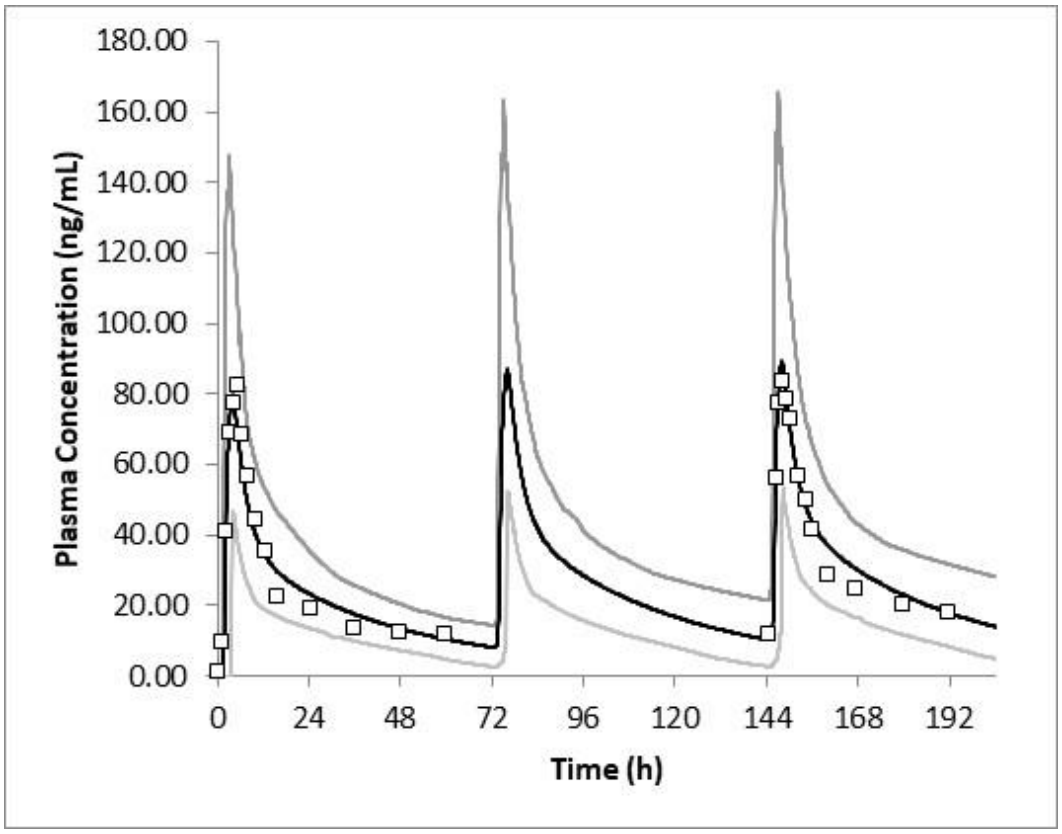


Figure S5. PBPK model simulated (lines; mean and 5th and 95th percentiles) plasma concentration-time profiles for ivermectin after 400 mcg/kg administered daily for 3 days.

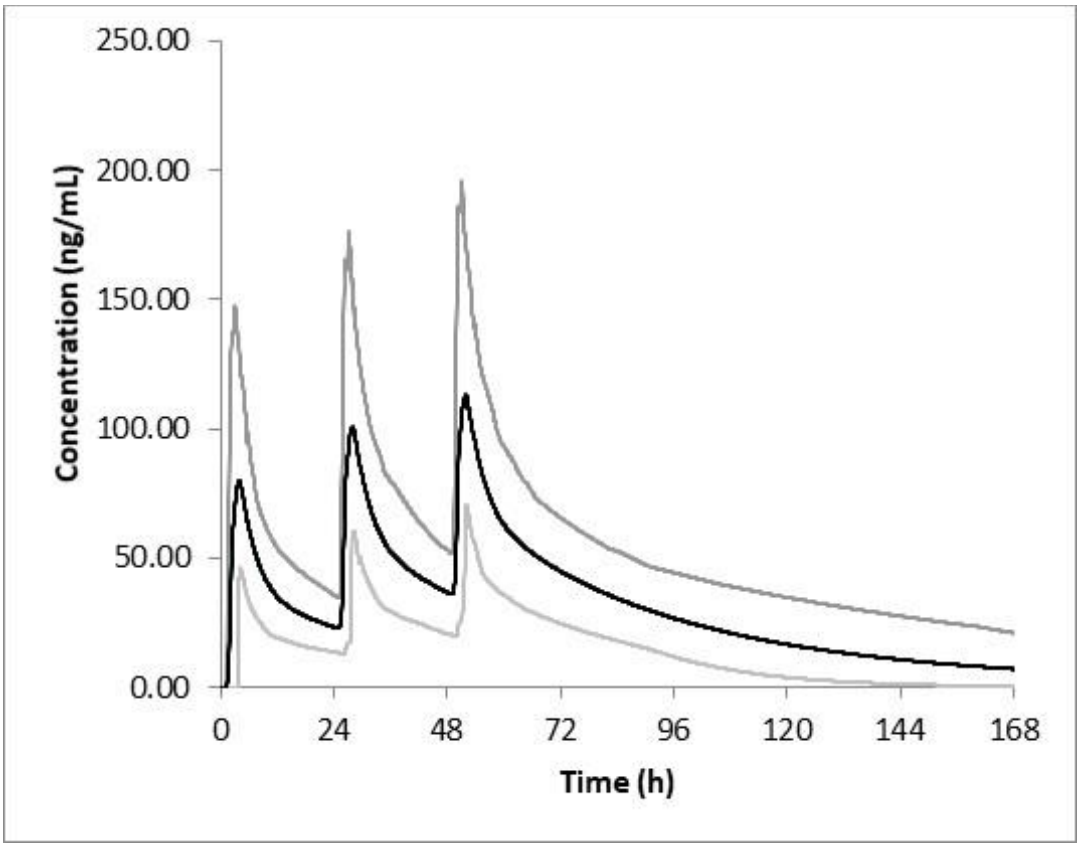
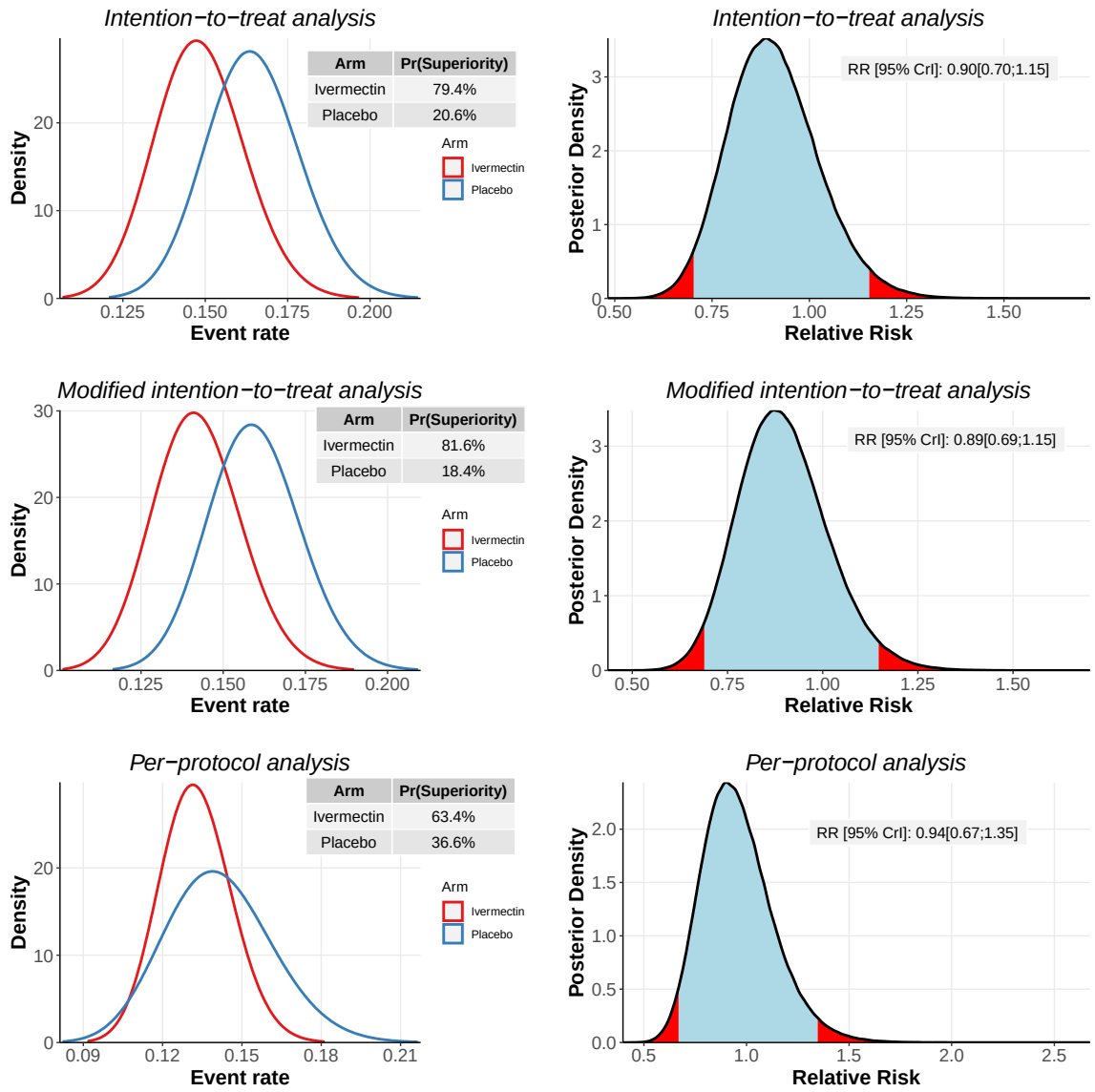


Figure S6: Probability of efficacy and Bayesian relative risk of Covid-19 hospitalization or extended emergency room observation for ivermectin vs. placebo



LEGEND: In the ivermectin group, 14.7% (100 of 679) of participants experienced a primary outcome event compared with 16.3% (111 of 679) in the placebo group. By intention-to-treat analysis, the Relative Risk was 0.90; 95% Bayesian Credible Interval [BCI]: 0.70 – 1.16) for ivermectin reducing the primary outcome of hospitalizations or prolonged emergency setting observations of >6 hours. The probability that the event rate was lower in the ivermectin group compared to placebo was 79.4% for the intention-to-treat population, 81.7% for the modified intention-to-treat population, and 63.4% in the per-protocol analysis.

Table S1: Components of the Primary Outcome of Ivermectin vs Placebo in the TOGETHER COVID-19 Trial

	Ivermectin	Placebo	Estimated treatment effect (95% BCI)
Hospitalized for COVID-19	78/679 (11.5%)	93/679 (13.7%)	0.84 (0.63 to 1.11)
Emergency room visit for greater than 6 hours	36/679 (5.3%)	31/679 (4.6%)	1.16 (0.73 to 1.85)

Table S2: Sub-Group Analysis for Hospitalization or Extended ER Observation Due to COVID-19

Factor	Subgroup	N_Placebo	N_Ivermectin	n_Placebo	n_Ivermectin	RR (95% BCI)
Age	<=50	347	335	39	38	1.01 (0.66, 1.53)
Age	>50	283	295	66	53	0.77 (0.56, 1.06)
Sex	Female	408	383	59	47	0.85 (0.59, 1.21)
Sex	Male	271	296	52	53	0.93 (0.66, 1.32)
BMI, kg/m^2	<30	333	345	48	38	0.77 (0.51, 1.14)
BMI, kg/m^2	>30	339	330	63	60	0.98 (0.71, 1.34)
Time from onset of symptoms	0-3 days	276	282	35	41	1.14 (0.76, 1.74)
Time from onset of symptoms	4-7 days	241	242	43	43	1.00 (0.68, 1.46)
Cardiovascular disease	N	407	397	58	53	0.94 (0.66, 1.32)
Cardiovascular disease	Y	272	282	53	47	0.86 (0.60, 1.22)
Lung disease	N	664	665	106	96	0.91 (0.70, 1.17)
Lung disease	Y	14	14	5	4	0.83 (0.28, 2.25)
Smoking status	Current	59	50	5	5	1.17 (0.38, 3.63)
Smoking status	Former	73	94	13	15	0.89 (0.46, 1.75)
Smoking status	Never	545	535	93	80	0.88 (0.67, 1.15)

Clinical Pharmacology Commentary

Throughout our trial, we have engaged with other clinical trialists working on COVID-19 and ivermectin. When we began this trial, we randomized patients to 1-day dosing of ivermectin, as that is the dose most commonly used for parasitic diseases. We responded to feedback of our dosing schedule by ivermectin advocacy groups and adapted our dosing to 3-days of ivermectin at a relatively high dose compared to most other trials after assuring the safety of using this extended ivermectin regimen.

Given the public interest in ivermectin and the support of its use by para-medical groups, we suspect there will be additional criticism that our dosing regimen was inadequate. Mechanistic hypotheses for ivermectin against SARS-CoV-2 encompass potential direct pharmacological effects (tracking directly with ivermectin systemic pharmacokinetics) or indirect pharmacological effects (time-delayed effects, not tracking directly with systemic pharmacokinetics) or combinations thereof. Ivermectin has a prolonged systemic half-life of 18 hours and longer residence times in tissues. We examined whether the timing of dosing affected outcomes and found no evidence of an effect as most events occur within the first 5 days post-randomization. Consequently, we were unable to identify signals for either a clinically meaningful direct or indirect pharmacological effect attributed to ivermectin. Available translational evidence, coupled with anticipated pharmacokinetic and pharmacodynamic variability, does not support clinical outcomes are likely to be different even with moderately higher ivermectin dose regimens or longer treatment durations.

Given the urgency of COVID-19, segments of the scientific community embraced the potential of ivermectin and sought to explain the mechanistic potential of ivermectin. As described in one recent review article, at least 20 potential mechanistic hypotheses supporting ivermectin's potential efficacy against SARS-CoV-2 had been postulated, many with little basis for translation to clinic.[5] These include examining direct action on SARS-Cov2, including interfering with interactions between the human protein complex importin (IMPα/β1) and virus proteins,[6] or interfering with cell entry of SARS-CoV-2 via ACE or TMPRESS2;[7,8] interference of a variety of host targets involved in viral replication,[9] invoking a broad range of anti-inflammatory and immunomodulatory effects including activity on interferons, toll-like receptors, interleukin 6 and JAK-STAT pathway,[10] as well as other host-related mechanisms.[8]

One study in SARS-COV-2 infected Syrian hamsters treated with a single 400 ug/kg subcutaneous dose of ivermectin confirmed lack of antiviral activity, but they reported improved clinical score and food-finding behavior, postulating beneficial cytokine/chemokine changes in hamsters.[11] Anticipated Cmax concentrations in hamsters in this experiment cited by the authors (80 ng/mL),[11] are lower than those expected in patients receiving 400 ug/kg once a day for 3 days (Cmax geometric mean 125.5 ng/mL; 90% CI 119.5 to 131.7 ng/mL using Simcyp Simulator Version 19 Release 1 under fasting conditions) in our trial, indicating a lack of clinically relevant translation to humans (see **Physiologically Based Pharmacokinetic (PBPK) modelling of ivermectin plasma exposure** below).

Physiologically Based Pharmacokinetic (PBPK) Modelling of Ivermectin Plasma Exposure

Input Parameters

Simulations of ivermectin plasma concentrations were performed using the Simcyp Simulator V19R1 (www.simcyp.com) and the input parameters indicated below (Table S3). Virtual individuals were generated using values and formulae describing demographic, anatomical and physiological variables representative of a population.[12]

Table S3: Input parameters for ivermectin PBPK model

Parameter	Value	Sources [if relevant]/Assumptions
Molecular weight [g/mol]	875.1	NA
Log P	5.83	NA
Compound type	Neutral	NA
B/P	1	Assumed
Fu	0.068	[13]
Absorption model	First order	NA
Fa	0.5	Assumed for fasted state based on clinical data [14]
Ka [1/h]	1	Fitted from clinical data [14,15]
Distribution model	Full PBMK model	NA
Vss [L/kg]	3.402	Predicted
Prediction method	Method 1	NA
Kp scalar	0.370	Fitted based on clinical data [14]
Clpo [L/h]	16.3	Clinical data [14]

Fasted versus Fed State for Ivermectin

The effect of food on exposure of ivermectin has been assessed formally in 2 clinical studies and in a POP-PK analysis of relevant clinical data (Table S4). Whilst a 2.5-fold increase in ivermectin exposure may be expected in younger healthy subjects, based on the data presented below, the food effect is likely to be attenuated in older subjects. Thus, a modest food-effect is expected in patients with COVID-19.

Table S4. Summary of reported studies to assess food effect on ivermectin

Dose (mg)	Subjects	Study design	Food effect	Reference
12	15 healthy subjects	POP-PK analysis of clinical data from 2 studies – one with a high-fat breakfast and the other in the fasted state	1.18 (95% CI: 1.10-1.67)	[16]
12	13 elderly Japanese subjects (73-95 years)	Food effect study (high fat meal) crossover	1.25 (90% CI: 1.09-1.43)	[17]
30	12 healthy subjects (21-45 years)	Dose escalation study including food effect arm (high-fat breakfast) – crossover	2.57 (95% CI: 2.16-3.05)	[18]

Verification of PBPK model at 30 mg (range: 347-594 mcg/kg) in a fasted state
Simulated plasma profiles of ivermectin were reasonably consistent with observed data reported previously following multiple oral doses of 30 mg[18], which is equivalent to 400 mcg/kg used in our study (Figure S4). On average, predicted Cmax and AUC (0,60) values were within 1.22- and 1.27-fold of the corresponding observed values.[18]

PBPK modelling of ivermectin with 0.4 mg/kg dosing for 3 days in a fasted state
Following oral administration of 400 mcg/kg daily for 3 days, simulated plasma profiles of ivermectin are shown in Figure S5. On Day 3, mean Cmax was 131 ng/mL (90% CI: 120-132) and mean AUC(0,Ƭ) was 1569 ng/mL (90% CI: 1444-1583).

Table S5: Representativeness of Study Participants

Category	Example
Disease under investigation	COVID-19
Special considerations related to	
Age	Infections and deaths increase with age, as 62% of infections are in people over 50, and 95% of deaths from COVID-19 are for those over 50 [19,20]
Sex	COVID-19 affects men more than women, with men 1.5x more than women likely to require hospitalization, 2x as likely to require intensive care, 1.7x more likely to die from the disease[19]
Race	COVID-19 affects different races disproportionately. However, this is thought to be due to socioeconomic factors. Compared with white Americans, African Americans 3.6x more likely to die, and Latinx 3.2x more likely to die, though mostly thought to be from socioeconomic factors [21]
Pre-existing conditions	89% of hospitalized patients had a pre-existing condition, most commonly hypertension (49.7%), obesity (48.3%), chronic lung disease (34.6%), diabetes mellitus (28.3%) and cardiovascular disease (27.8%).[19]
Other considerations	Most of the data gathered on the different effects of COVID-19 on different groups were gathered from the US and Europe, as data was lacking for Brazil. Some of these aspects, especially the differences in race, may not translate directly to Brazil as many of the differences were related to socioeconomic factors in the US.
Overall representativeness of the trial	The participants in the trial were balanced between male and female (41.8% to 58.2%). Participants were asked their age, sex, race and pre-existing conditions during the screening visit. The proportion of race was 95.2% mixed race, 0.9% black/African American, and 2.9% unknown. Our age distribution was also evenly split, with 53.8% of participants < 50, while 46.2% were >= 50. The study had a higher representation of females, which represented the course of the pandemic at that time. The distribution of females and males was split evenly between the ivermectin arm and placebo.

A search of Pubmed was done to determine how COVID-19 affects people of different ages, sex, race and pre-existing conditions.

Table S6: Adverse Events by Grade, MedDRA Type and Treatment Group

	Ivermectin			Placebo		
Characteristic	**Grade 1 or 2**, N = 82[1]	**Grade 3 or 4**, N = 60[1]	**Grade 5**, N = 21[1]	**Grade 1 or 2**, N = 105[1]	**Grade 3 or 4**, N = 71[1]	**Grade 5**, N = 24[1]
Standard of care term (MedDRA)						
Gastrointestinal disorders	3 (3.7%)	0 (0%)	0 (0%)	6 (5.7%)	1 (1.4%)	0 (0%)
General disorders and administration site conditions	31 (38%)	4 (6.7%)	1 (4.8%)	39 (37%)	1 (1.4%)	1 (4.2%)
Immune system disorders	1 (1.2%)	0 (0%)	0 (0%)	0	0	0
Infections and infestations	15 (18%)	54 (90%)	20 (95%)	28 (27%)	64 (90%)	21 (88%)
Injury, poisoning and procedural complications	1 (1.2%)	0 (0%)	0 (0%)	0	0	0
Metabolism and nutrition disorders	2 (2.4%)	1 (1.7%)	0 (0%)	2 (1.9%)	0 (0%)	0 (0%)
Musculoskeletal and connective tissue disorders	7 (8.5%)	1 (1.7%)	0 (0%)	5 (4.8%)	1 (1.4%)	0 (0%)
Nervous system disorders	3 (3.7%)	0 (0%)	0 (0%)	5 (4.8%)	0 (0%)	0 (0%)
Psychiatric disorders	2 (2.4%)	0 (0%)	0 (0%)	0	0	0
Reproductive system and breast disorders	1 (1.2%)	0 (0%)	0 (0%)	0	0	0
Respiratory, thoracic and mediastinal disorders	11 (13%)	0 (0%)	0 (0%)	16 (15%)	1 (1.4%)	0 (0%)
Skin and subcutaneous tissue disorders	1 (1.2%)	0 (0%)	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)
Vascular disorders	4 (4.9%)	0 (0%)	0 (0%)	1 (1.0%)	1 (1.4%)	0 (0%)
Cardiac disorders	0	0	0	0 (0%)	1 (1.4%)	2 (8.3%)
Ear and labyrinth disorders	0	0	0	1 (1.0%)	0 (0%)	0 (0%)
Hepatobiliary disorders	0	0	0	0 (0%)	1 (1.4%)	0 (0%)
Renal and urinary disorders	0	0	0	1 (1.0%)	0 (0%)	0 (0%)

[1]n (%)

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