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High-dose ivermectin for early treatment of COVID-19 (COVER study): a randomised, double-blind, multicentre, phase II, dose-finding, proof-of-concept clinical trial

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ABSTRACT

High concentrations of ivermectin demonstrated antiviral activity against SARS-CoV-2 in vitro. The aim of this study was to assess the safety and efficacy of high-dose ivermectin in reducing viral load in individuals with early SARS-CoV-2 infection. This was a randomised, double-blind, multicentre, phase II, dose-finding, proof-of-concept clinical trial. Participants were adults recently diagnosed with asymptomatic/oligosymptomatic SARS-CoV-2 infection. Exclusion criteria were: pregnant or lactating women; CNS disease; dialysis; severe medical condition with prognosis <6 months; warfarin treatment; and antiviral/chloroquine phosphate/hydroxychloroquine treatment. Participants were assigned (ratio 1:1:1) according to a randomised permuted block procedure to one of the following arms: placebo (arm A); single-dose ivermectin 600 μg/kg plus placebo for 5 days (arm B); and single-dose ivermectin 1200 μg/kg for 5 days (arm C). Primary outcomes were serious adverse drug reactions (SADRs) and change in viral load at Day 7. From 31 July 2020 to 26 May 2021, 32 participants were randomised to arm A, 29 to arm B and 32 to arm C. Recruitment was stopped on 10 June because of a dramatic drop in cases. The safety analysis included 89 participants and the change in viral load was calculated in 87 participants. No SADRs were registered. Mean (S.D.) $\log_{10}$ viral load reduction was 2.9 (1.6) in arm C, 2.5 (2.2) in arm B and 2.0 (2.1) in arm A, with no significant differences ($P = 0.099$ and 0.122 for C vs. A and B vs. A, respectively). High-dose ivermectin was safe but did not show efficacy to reduce viral load.

1. Introduction

The vaccine roll-out has certainly played a key role in the fight against COVID-19 (coronavirus disease 2019). However, the emergence of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) variants and the (slow) pace of vaccination have

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hampered the expected drop in cases in most parts of the world [1,2]. Thus, the pandemic is still hitting hard and antiviral drugs, preferably at low cost and widely available, are still badly needed.

Initially, research has been oriented towards drug repurposing, which is time-saving compared with new drug discovery [3]. Several compounds have been tested against SARS-CoV-2 in vitro and subsequently in vivo, including drugs not primarily used as antiviral agents. In particular, chloroquine/hydroxychloroquine and ivermectin were of interest for use in low- and middle-income countries as these drugs are widely available (for treatment of parasitic infections) and inexpensive [3]. Although recent evidence has globally shifted attention towards novel (and much more expensive) ad hoc medications [4–7], interest in a possible role for cheaper and older compounds remains.

In March 2020, Australian researchers demonstrated that ivermectin, a drug used for decades to combat parasitic infections, had antiviral activity against SARS-CoV-2 in vitro in Vero cell cultures, virtually reducing the viral load to zero in 48 h [8]. This sparked considerable interest in this 'old' drug as a potential cure for COVID-19, resulting on one hand in a consistent number of clinical trials testing ivermectin efficacy, and on the other in an uncontrolled procurement of the drug for self-treatment [9]. However, a plasma concentration of ivermectin compatible with the IC_{50} (half maximal inhibitory concentration) found in vitro (~2.5 μM, equivalent to 2190 ng/mL) is far from being achievable with the usual doses of this drug [10] (40–80 ng/mL after a dose of 200–400 μg/kg). Based on this, we would expect ivermectin to show neither clinical nor virological efficacy against COVID-19 unless higher doses are administered.

The aim of this study was to assess the efficacy and safety of high doses of ivermectin (namely doses of 600 μg/kg and 1200 μg/kg for 5 consecutive days) for the treatment of SARS-CoV-2 infection. The doses were chosen based on the following considerations: (i) according to the reported pharmacokinetic (PK) profile in plasma [11,12], it can be supposed that repeated daily administrations result in drug accumulation, with predicted plasma levels only slightly higher than those observed with single administration of 2000 μg/kg in fasted state (248 ng/mL), which was well tolerated [11]; and (ii) studies in mammals [13,14] showed much higher ivermectin levels in pulmonary tissue than in plasma.

The primary objectives of this study were to define: (i) whether ivermectin, administered at two different high dosages, is safe in participants with initial, asymptomatic or oligosymptomatic SARS-CoV-2 infection; and (ii) whether ivermectin, administered at the dosage(s) found to be safe decreases the viral load of SARS-CoV-2 at Day 7.

2. Materials and methods

2.1. Study design

The COVER study was a randomised, investigator-initiated, double-blind, multicentre, phase II, dose-finding, proof-of-concept clinical trial carried out in outpatients consecutively diagnosed in four sites in Italy. The study protocol (available in the Supplementary material) was approved by the national Ethics Committee of INMI–Spallanzani in Rome, which is competent for all COVID-19 trials in Italy (resolution 139/2020 of 28 May 2020) and by the Italian drug agency AIFA (resolution 136BIS/2020 of 18 May 2020). The protocol was also registered with ClinicalTrials.gov (NCT04438850). The study complied with the Declaration of Helsinki and was performed in accordance with Good Clinical Practice guidelines. All participants provided written informed consent.

2.2. Participants and study sites

Participants were adult (≥18 years) subjects newly diagnosed with SARS-CoV-2 infection by real-time PCR analysis of nasopharyngeal swabs, not requiring hospitalisation or oxygen supplementation (COVID-19 severity score <3 [15]) and providing their informed consent to the study. The main exclusion criteria were: pregnant or lactating women; known central nervous system disease; participants receiving dialysis; any severe medical condition with a prognosis of <6 months; warfarin treatment; antiviral treatment; and chloroquine phosphate or hydroxychloroquine treatment.

2.3. Randomisation and masking

The study arms were as follows: placebo arm (arm A); single-dose ivermectin 600 μg/kg plus placebo for 5 days (arm B); and single-dose ivermectin 1200 μg/kg for 5 days (arm C).

Participants were randomly assigned by a centralised computer system to one of the three arms with an allocation ratio of 1:1:1. The study biostatistician prepared the sequence of treatments, generated using SAS 9.4 software according to a randomised permuted blocks procedure. The treatment ID was obtained through REDCap, used as a web-based clinical data management system for the study. Following randomisation, the treatment ID and the patient's weight were communicated to the hospital pharmacist who was in charge to prepare the study treatment according to the randomisation list. In order to keep investigators and participants blinded, the number of tablets to be administered was the same, irrespective of the study arm in which the participant was randomised. Placebo tablets were identical in appearance and taste to ivermectin. Also, the staff in charge of the laboratory analyses were blinded.

Following randomisation, participants were provided the daily therapy in five packs, numbered 1 to 5 with the respective dates and labelled with the randomisation code. The division into single doses and the labelling were done by the study pharmacists under controlled conditions. The daily doses were self-administered on an empty stomach with water.

2.4. Study procedures

Starting with Day 1 (day of first dose), investigators contacted the participants daily by phone until Day 5 in order to check for the correct intake of the drug and for the occurrence of any adverse events (AEs) based on a pre-specified list. At Day 7, the participant was re-assessed at an in-person visit and a nasopharyngeal swab was performed and examined locally by RT-PCR, then stored at –80°C until the final (centralised) analysis. Full blood count and transaminases were also checked locally. Further onsite visits were scheduled on Day 14 (in all similar to Day 7) and Day 30 (final follow-up visit). Nasopharyngeal swabs were performed if positive at previous visit, and the leftover material was stored as above. Unscheduled visits for any reason were also foreseen. Participants were provided with a dedicated phone number so that they could contact the investigators in case of need.

AEs were assessed for severity according to National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) grading v.4.03, and the causal relationship to the study treatment or to SARS-CoV-2 infection was established by the study physician and recorded in the electronic case report form (eCRF).

Serious adverse events (SAEs) or serious adverse drug reactions (SADRs) had to be immediately (not later than 24 h) reported to the sponsor as well as to the Study Safety Desk. The same applied to suspected unexpected serious adverse reactions (SUSARs).

2.5. Study outcomes

Primary outcomes were: (i) number of SADRs; and (ii) change in viral load at Day 7 with respect to baseline.

Secondary outcomes were: (i) trend over time in quantitative viral load at Days 7, 14 and 30; (ii) time to clinical resolution (TCR) (if symptomatic); (iii) proportion of participants with virological clearance at Days 14 and 30; (iv) hospitalisation rate; and (v) COVID-19 severity score at Days 14 and 30.

2.6. Statistical analysis

The statistical study design was conceived in two steps.

Step 1 aimed at testing the safety of the two experimental arms, in terms of SADRs occurrence, on the first 60 evaluable participants (20 per arm). If the experimental arm failed to be demonstrated as safe, the allocation of participants was planned to be interrupted. At the same time, interim analyses on efficacy and futility were planned comparing control and experimental arms. Futility and efficacy criteria for prematurely stopping the trial are detailed in the study protocol.

Step 2 aimed at testing the efficacy of experimental arms, considered safe at first stage, compared with the control arm in terms of viral load decrease.

The primary safety endpoint was the occurrence of a SADR. According to A'Hern's [16] single-stage design, setting a type I error rate of 10% and power of 80% and hypothesising that the true toxicity rate (SADRs) was 5%, 20 participants in each experimental arm were needed to test the null hypothesis that the toxicity rate was 30% against a one-sided alternative.

The primary efficacy endpoint was the difference in viral load decline from baseline to Day 7. Viral load was measured as $\log_{10}$ of genome copies per μL, ascertained by droplet digital quantitative PCR (dd-PCR) [17,18]. To et al. [19] reported a difference in mean viral load decline of 1.05 $\log_{10}$ copies [standard deviation (S.D.) 0.69 $\log_{10}$ copies]. The desired difference in decrease between each experimental group and control was at least 0.47 $\log_{10}$ copies/mL (effect size $\Delta = 0.68$, considered to be of moderate-large magnitude according to Cohen [20]). According to these hypotheses, with 34 participants per arm the study had a power of 80% to detect a difference between control and experimental arms, at 0.025 α level, one-sided. Therefore, if both experimental treatments proceeded to step 2 and were compared with the control group, the total number of participants to be analysed had to be 102. Considering a potential 20% loss to follow-up, including missing or inadequate specimens, it was planned to enrol 129 participants.

Data were analysed according to the Statistical Analysis Plan (Supplementary material) using SAS 9.4 software. The primary safety analysis was performed on the safety analysis set including participants who received at least one dose of study treatment.

Analysis of the primary efficacy endpoint was performed on the evaluable analysis set, considering all participants as originally assigned to the treatment arms without major violations of eligibility criteria and having the viral load evaluable at Day 7.

A per-protocol (PP) analysis set was defined including only participants who took the allocated treatment as specified in the protocol for 5 days in order to check for consistency with the primary analysis.

2.6.1. Analysis of primary outcomes

The proportion of participants in each experimental group experiencing at least one SADR was described by means of frequency and percentage.

The mean of the differences in viral load decline from baseline to Day 7 between each experimental group and the control group was described by standard summary measures for continuous data and was compared by Student's *T*-test and Wilcoxon test (according to Shapiro–Wilk test for normality).

2.6.2. Analysis of secondary outcomes

A full description of the statistical analyses of all secondary outcomes is reported in the Statistical Analysis Plan (Supplementary material). Assessment of the tolerability profile was mainly based on adverse drug reactions and the frequency and nature of SAEs. The proportion of participants in each group experiencing AEs was compared by χ^2 test (or Fisher's exact test when appropriate). Generalised longitudinal mixed models were used to analyse the trend over time in viral load at the different time points. TCR was calculated as the time from randomisation to clinical resolution or death. TCR was analysed with Cox regression models and was described by the Kaplan–Meier method. The proportion of participants with virological clearance was described by frequencies and proportions with their relative exact 95% confidence interval (CI). The χ^2 test was used to test the proportion of participants with virological clearance at Day 14, and Fisher's exact test was used at Day 30.

2.6.3. Ancillary study on pharmacokinetics

Measurement of plasma concentrations of ivermectin was included as an ancillary study to inform on the maximal drug levels and drug accumulation following repeated doses, on the interindividual variability and, possibly, on the association between blood drug concentration and clinical endpoints. In fact, a high variability in absorption has been described [11]. Peak plasma concentrations (C_{max}) are observed at approximately 4 h after dosing, with an elimination half-life of 18–35 h [11,12]. For these reasons and for feasibility considerations, it was planned to collect blood samples in a subset of participants at three timepoints: just before and 4 h and 48 h after the fifth dose. Plasma aliquots were stored at –20°C (or lower temperature) until analysis.

Ivermectin was measured by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) at the laboratory of the IRCCS Sacro Cuore Don Calabria Hospital using a method validated according to the European Medicines Agency (EMA) guidelines. Details on the procedure are reported in the study protocol.

2.7. Role of the funding sources

The sponsor was IRCCS Sacro Cuore Don Calabria Hospital, which received funds for this trial from the Italian Ministry of Health in the framework of 'Ricerca corrente'. Tablets of 9 mg ivermectin and placebo were donated by Insud Pharma (Madrid, Spain). The funders had no role in study design, data collection, analysis and interpretation, or writing of the report.

3. Results

From 31 July 2020 to 26 May 2021, 93 participants were randomised to the three study arms: 32 to arm A; 29 to arm B; and 32 to arm C. IRCCS Ospedale Sacro Cuore Don Calabria of Negrar contributed 79 participants, Ospedale 'Luigi Sacco' ASST Fatebenefratelli Sacco of Milano contributed 8 participants, Azienda Ospedaliera-Universitaria–Policlinico S. Orsola-Malpighi of Bologna contributed 4 participants and Azienda Provinciale Servizi Sanitari Trento–Ospedale of Rovereto contributed 2 participants.

In March 2021, at the interim analysis performed after the enrolment of 64 participants, no SADRs were observed. Furthermore, none of the experimental arms were stopped for efficacy or futility.

Recruitment was stopped on 10 June 2021 because of a dramatic drop in cases. The last follow-up visit was carried out on 29 June 2021.

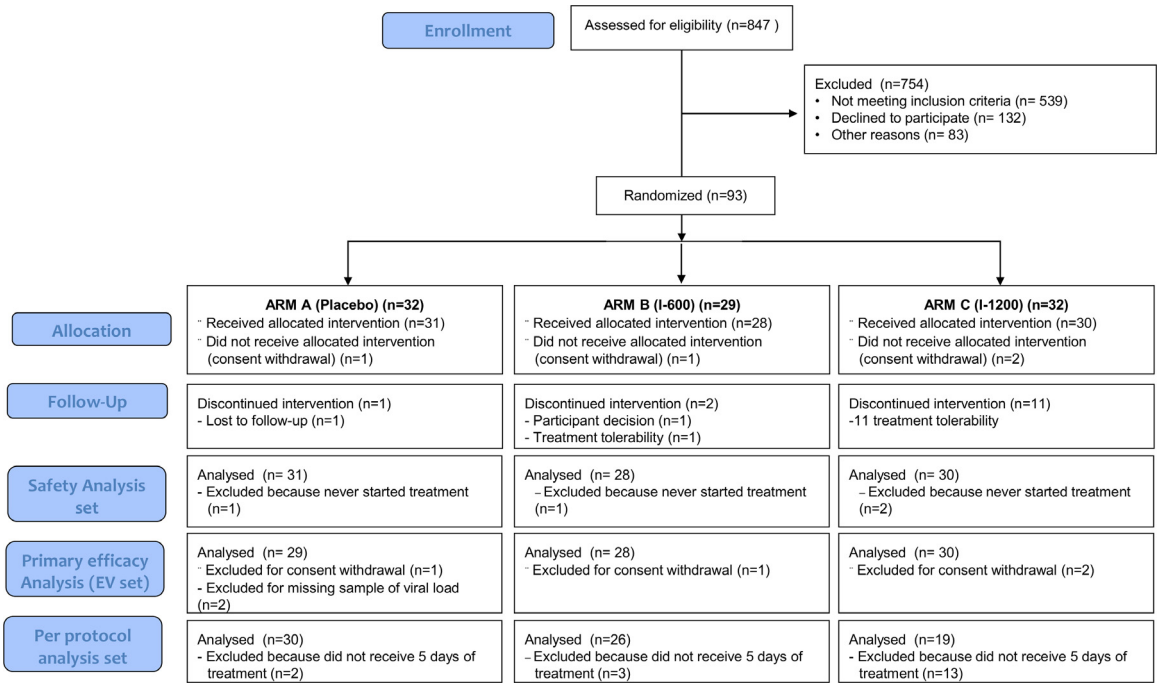


Fig. 1. Study flow chart. Information regarding arm of randomisation, number of subjects screened and enrolled, and detailed reasons for missing inclusion are presented.

Detailed information on screened participants and the reasons for missing inclusions were retrieved from the screening log handled by the centres and is summarised in the study flow chart (Fig. 1; Supplementary Table S1). This information was provided by all centres but one (Rovereto, which contributed two randomised participants). Among the other centres, 845 participants were screened and 91 (10.8%) were randomised. The main reasons for exclusion were infection not recent (56.9%), patient refusal (15.4%), severity score >2 (7.8%) and organisational reasons (7.2%). The 93 participants were randomly assigned to the treatment arms. Of the 93 participants, 4 never started the treatment and withdrew consent and the other 89 received at least 1 day of study treatment and were therefore included in the analysis of the primary endpoints. Moreover, 2 participants (both in arm A) had a missing sample for viral load at Day 7, therefore 87 participants (93.5%) were included in the primary endpoint for efficacy. Moreover, 75 participants (80.6%) received 5 days of treatment and were included in the PP analysis set.

The main baseline demographic and clinical characteristics of the study population are summarised in Table 1 for all randomised participants and in Supplementary Table S2 for participants included in the evaluable analysis set. Male sex was more represented (58.1%). The median age was 47.0 years [interquartile range (IQR) 31.0–58.0]. The majority of participants were European (96.8%). Co-morbidities were reported by one-third of participants. Eighty participants (86.0%) were symptomatic with a median duration of 4 days, with cough being the most frequent symptom, followed by fever and fatigue. The severity score was 1 for 78 participants (83.9%) and 2 for 15 participants (16.1%).

Details of the main physical findings, baseline laboratory examinations and concomitant treatments are summarised in Supplementary Tables S3–S5.

Summary of treatment compliance is reported in Table 2. Fourteen participants (15.1%) discontinued treatment: 1 (3.1%) in arm A; 2 (6.9%) in arm B; and 11 (34.4%) in arm C. The interruptions in arm C were all due to tolerability. Seven participants received only 1 day of treatment, three participants received 2 days and 3 days, respectively, and one participant received 4 days (Supplementary Table S6).

No SADRs were observed in any of the study groups. Results for viral load ($\log_{10}$) at Day 7 versus baseline are summarised in Table 3. The mean reduction in viral load was 2.9 (SD 1.6) in arm C versus 2.5 (SD 2.2) in arm B and 2.0 (SD 2.1) in arm A. The observed effect size (versus arm A) was 0.48 for arm C and 0.21 for arm B. Differences were not normally distributed (Shapiro–Wilk test P-value <0.0001 for both comparisons), thus Wilcoxon exact test was also performed. The differences were not significant ($P = 0.099$ and 0.122 for arm C vs. arm A and arm B vs. arm A, respectively). Results according to PP analysis, summarised in Supplementary Table S7, are superimposable.

The trend over time of the quantitative viral load is summarised in Supplementary Tables S8 and S9. In both non-adjusted and multivariate mixed model, a significant interaction was found between arm C and Day 7 (P-value = 0.035 and 0.036, respectively). TCR was described in symptomatic participants (80 participants). The median TCR was 14 days (IQR 13–30 days), 29 days (IQR 13.5–32.0 days) and 14 days (IQR 7–37 days) for arms A, B and C, respectively. Kaplan–Meier curves for TCR comparisons between arms are reported in Supplementary Fig. S1. Differences between arms were not significant [hazard ratio (HR) = 0.69, 95% CI 0.36–1.32 ($P = 0.262$) and HR = 0.79, 95% CI 0.42–1.47 ($P = 0.456$) for arm B vs. arm A and for arm C vs. arm A, respectively] (Supplementary Table S10). The proportion of participants with virological clearance at Days 14 and 30 is summarised in Supplementary Table S11. Within 14 days, 59% (95% CI 22.4–61.2%), 70% (95% CI 13.2–52.9%) and 58% (95% CI 23.4–63.1%) of participants in arms A, B and C achieved virological clearance.

As an exploratory analysis, the same proportion for Day 7 was also added. No significant difference between arms was observed at any of the time points. The hospitalisation rate was 3/30 (10.0%) for arm C, 1/29 (3.4%) for arm B and 0 for arm A. Regarding the COVID-19 severity score, at Day 14 all participants had a score of 1, except for two participants in arm B with severity scores of 2 and 3. At Day 30 all participants had a severity score of 1.

Table 1
Demographic and baseline characteristics of the study participants, overall and by study arm

Characteristic	Arm A (N = 32)	Arm B (N = 29)	Arm C (N = 32)	Overall (N = 93)
Age (years) [median (IQR)]	50.0 (26.0–57.0)	47.0 (31.0–62.0)	44.5 (31.0–55.5)	47.0 (31.0–58.0)
Female sex [n (%)]	17 (53.1)	14 (48.3)	8 (25.0)	39 (41.9)
Weight (kg) [median (IQR)]	69.0 (62.5–74.0)	72.0 (61.0–84.0)	79.0 (70.5–85.0)	72.0 (63.0–82.0)
Height (cm) [median (IQR)]	170.0 (164.5–178.0)	170.0 (167.0–175.0)	173.0 (170.0–180.0)	170.0 (167.0–178.0)
Nation of origin [n (%)]				
European	29 (90.6)	29 (100.0)	32 (100.0)	90 (96.8)
Extra-European	3 (9.4)	0 (0.0)	0 (0.0)	3 (3.2)
Setting of baseline visit [n (%)]				
Home	27 (84.4)	24 (82.8)	23 (71.9)	74 (79.6)
Hospital emergency room	3 (9.4)	2 (6.9)	6 (18.8)	11 (11.8)
Hospital outpatient ambulatory care	1 (3.1)	2 (6.9)	3 (9.4)	6 (6.5)
Other	1 (3.1)	1 (3.4)	0 (0.0)	2 (2.2)
Co-morbidities [n (%)]	8 (25.0)	11 (37.9)	12 (37.5)	31 (33.3)
Respiratory	0 (0.0)	4 (36.4)	2 (16.7)	6 (19.4)
Cardiovascular	7 (87.5)	7 (63.6)	8 (66.7)	22 (71.0)
Diabetes	2 (25.0)	0 (0.0)	1 (8.3)	3 (9.7)
Time from diagnosis to randomisation (days) [median (IQR)]	1.0 (0.0–1.0)	1.0 (1.0–2.0)	1.0 (0.5–2.0)	1.0 (1.0–2.0)
Symptoms [n (%)]	27 (84.4)	24 (82.8)	29 (90.6)	80 (86.0)
Cough	10 (37.0)	9 (37.5)	16 (55.2)	35 (43.8)
Pyrexia (>37.5°C)	9 (33.3)	8 (33.3)	16 (55.2)	33 (41.3)
Fatigue	10 (37.0)	6 (25.0)	10 (34.5)	26 (32.5)
Myalgia	6 (22.2)	3 (12.5)	13 (44.8)	22 (27.5)
Headache	7 (25.9)	4 (16.7)	9 (31.0)	20 (25.0)
Anosmia	4 (14.8)	4 (16.7)	9 (31.0)	17 (21.3)
Infective rhinitis	4 (14.8)	10 (41.7)	1 (3.4)	15 (18.8)
Dysgeusia	4 (14.8)	2 (8.3)	2 (6.9)	8 (10.0)
Oropharyngeal pain	2 (7.4)	4 (16.7)	3 (10.3)	9 (11.3)
Diarrhoea	0 (0.0)	3 (12.5)	2 (6.9)	5 (6.3)
Asthenia	3 (11.1)	0 (0.0)	2 (6.9)	5 (6.3)
Other [a]	7 (25.9)	5 (20.8)	3 (10.3)	15 (18.8)
Days with symptoms [median (IQR)]	4.0 (2.0–6.0)	4.0 (3.0–5.0)	4.0 (3.0–6.0)	4.0 (3.0–5.5)
COVID-19 severity score [n (%)]				
1, no limitation of activities	27 (84.4)	24 (82.8)	27 (84.4)	78 (83.9)
2, limitation of activities	5 (15.6)	5 (17.2)	5 (15.6)	15 (16.1)
SARS CoV-2 vaccine [n (%)]	1 (3.1)	1 (3.4)	0 (0.0)	2 (2.2)

IQR, interquartile range; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
NOTE: Arm A, placebo; arm B, ivermectin 600 μg/kg + placebo for 5 days; and arm C, ivermectin 1200 μg/kg for 5 days.
[a] Includes back pain, arthralgia, nausea, odynophagia, dyspnoea, ageusia, decreased appetite, ear pain, hyperaesthesia, musculoskeletal pain, pharyngitis and pruritic rash.

Table 2
Summary of treatment compliance, overall and by study arm

	Arm A (N = 32)	Arm B (N = 29)	Arm C (N = 32)	Overall (N = 93)
Treatment never started [n (%)]	1 (3.1)	1 (3.4)	2 (6.3)	4 (4.3)
Reason treatment for never started [n (%)]				
Consent withdrawal	1 (100.0)	1 (100.0)	2 (100.0)	4 (100.0)
Treatment discontinued [n (%)]	1 (3.1)	2 (6.9)	11 (34.4)	14 (15.1)
Reason for discontinuation [n (%)]				
Treatment tolerability	0 (0.0)	1 (50.0)	11 (100.0)	12 (85.7)
Participant decision	0 (0.0)	1 (50.0)	0 (0.0)	1 (7.1)
Lost to follow-up	1 (100.0)	0 (0.0)	0 (0.0)	1 (7.1)
Treatment completed [n (%)]	30 (93.8)	26 (89.7)	19 (59.4)	75 (80.6)

NOTE: Arm A, placebo; arm B, ivermectin 600 μg/kg + placebo for 5 days; and arm C, ivermectin 1200 μg/kg for 5 days.

The maximum grade of each AE per participant is summarised in Table 4 and in Supplementary Table S12. Overall, 229 AEs were recorded, 46 in arm A (20.1%), 69 in arm B (30.1%) and 114 in Arm C (49.8%). Only two AEs were grade 3 and no AEs of grades 4 or 5 were observed. The most reported AE concerned transient eye disorders [21/30 participants (70.0%) in arm C, 13/28 participants (46.4%) in arm B and 1/31 participants (3.2%) in arm A], followed by nervous system disorders, fatigue and gastrointestinal symptoms.

Four AEs were recorded as serious (SAEs): in all cases they required hospitalisation for worsening of the disease with no causal relationship to the study drug. Three SAEs occurred in arm C, 1 in arm B and none in arm A. All events resolved.

3.1. Ancillary study on pharmacokinetics

Concentrations of ivermectin were measured, in blind, in the plasma of 15 participants. The results are summarised in Fig. 2. The drug was not detectable in the plasma of five participants of arm A (placebo). Seven participants belonged to arm B and three to arm C, and in two of the latter the third time point was 72 h after the fifth dose. At T4 (4 h after the fifth dose), i.e. the time corresponding to the published C_{max}, the concentration ranged from 79.2 to 414.8 ng/mL for arm B and from 337.1 to 1082.0 ng/mL for arm C. The area under the concentration–time curve (AUC), calculated for the observed 0–48 h period (that is AUC_{96-144} if taking into account that measurements were done after the fifth dose)

Table 3
Primary efficacy endpoint of viral load in the evaluable analysis set, overall and by study arm

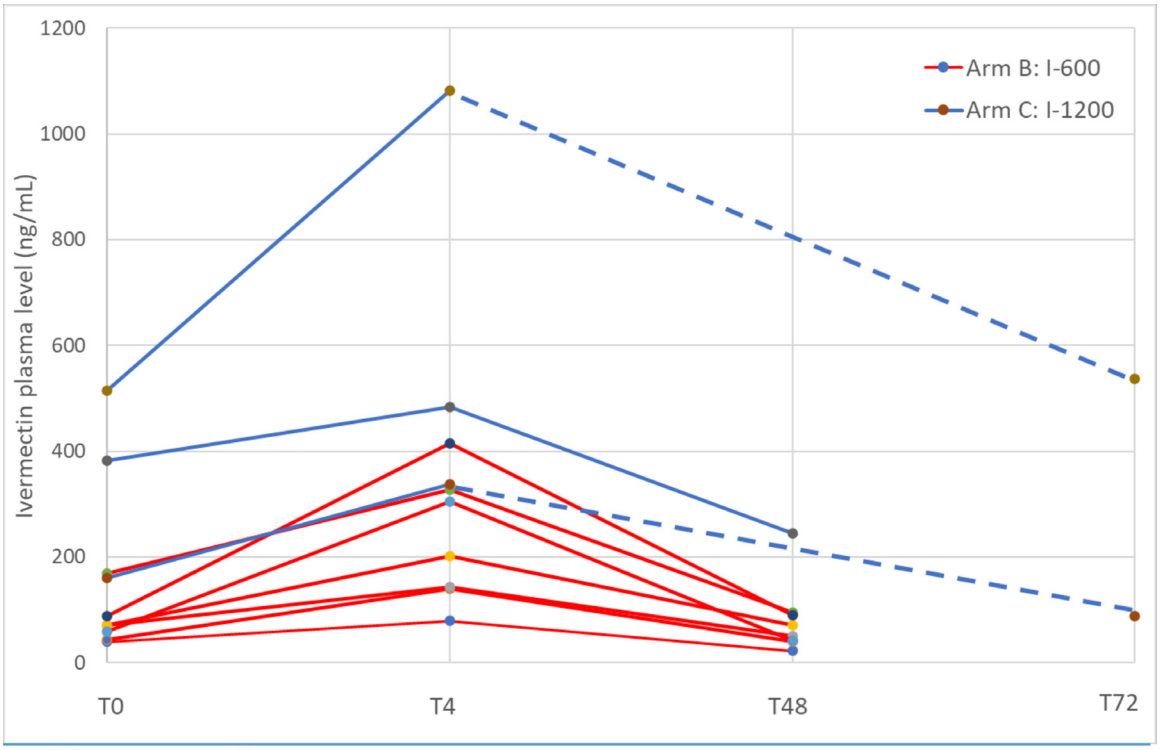
	Arm A ($N = 29$)	Arm B ($N = 28$)	Arm C ($N = 30$)	Overall ($N = 87$)
SARS-CoV-2 viral load at baseline (log_{10})				
Mean ± S.D.	4.3 ± 1.3	4.3 ± 1.2	4.5 ± 1.2	4.4 ± 1.2
Median (IQR)	4.4 (3.5–5.2)	4.3 (3.8–5.1)	4.4 (4.0–5.2)	4.4 (3.8–5.2)
Range	1.7–6.6	2.3–6.6	2.1–7.0	1.7–7.0
Missing	1	0	1	2
SARS-CoV-2 viral load at Day 7 (log_{10})				
Mean ± S.D.	2.2 ± 1.5	1.9 ± 1.6	1.6 ± 1.2	1.9 ± 1.4
Median (IQR)	2.3 (1.1–2.8)	1.5 (0.9–2.5)	1.6 (0.9–2.5)	1.7 (0.9–2.7)
Range	0.0–6.7	0.0–5.2	0.0–5.4	0.0–6.7
Differences in viral load decline from baseline to 7 days				
Mean ± S.D.	2.0 ± 2.1	2.5 ± 2.2	2.9 ± 1.6	2.5 ± 2.0
Median (IQR)	2.6 (1.6–3.2)	3.1 (2.3–4.1)	3.1 (1.8–4.1)	2.8 (1.7–3.7)
Range	–4.8 to 4.9	–2.9 to 4.9	–0.2 to 5.5	–4.8 to 5.5
Missing	1	0	1	2
Effect size				
Arm B vs. arm A				0.21
Arm C vs. arm A				0.48
Shapiro–Wilk test *P*-value (test for normal distribution)				
Arm B vs. arm A				<0.0001
Arm C vs. arm A				<0.0001
Wilcoxon exact *P*-value				
Arm B vs. arm A				0.122
Arm C vs. arm A				0.099
T-test *P*-value				
Arm B vs. arm A				0.429
Arm C vs. arm A				0.078

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S.D., standard deviation; IQR, interquartile range.
NOTE: Arm A, placebo; arm B, ivermectin 600 μg/kg + placebo for 5 days; and arm C, ivermectin 1200 μg/kg for 5 days.

Table 4
Adverse events in the safety analysis set

Adverse event/arm	Adverse events [*n* (%)] Grade 0	Grade 1	Grade 2	Grade 3	χ^2 for trend arm B vs. arm A	χ^2 for trend arm C vs. arm A
Eye disorders [a]					<0.0001	<0.0001
Arm A ($N = 31$)	30 (96.8)	1 (3.2)	0 (0.0)	0 (0.0)		
Arm B ($N = 28$)	15 (53.6)	12 (42.9)	1 (3.6)	0 (0.0)		
Arm C ($N = 30$)	9 (30.0)	16 (53.3)	5 (16.7)	0 (0.0)		
Gastrointestinal disorders [b]					0.506	0.029
Arm A ($N = 31$)	25 (80.6)	5 (16.1)	1 (3.2)	0 (0.0)		
Arm B ($N = 28$)	21 (75.0)	5 (17.9)	2 (7.1)	0 (0.0)		
Arm C ($N = 30$)	17 (56.7)	8 (26.7)	5 (16.7)	0 (0.0)		
General disorders and administration site conditions [c]					0.185	0.023
Arm A ($N = 31$)	17 (54.8)	13 (41.9)	1 (3.2)	0 (0.0)		
Arm B ($N = 28$)	10 (35.7)	17 (60.7)	1 (3.6)	0 (0.0)		
Arm C ($N = 30$)	8 (26.7)	19 (63.3)	3 (10.0)	0 (0.0)		
Infections and infestations [d]					0.293	0.080
Arm A ($N = 31$)	31 (100)	0 (0.0)	0 (0.0)	0 (0.0)		
Arm B ($N = 28$)	27 (96.4)	0 (0.0)	0 (0.0)	1 (3.6)		
Arm C ($N = 30$)	27 (90.0)	0 (0.0)	2 (6.7)	1 (3.3)		
Musculoskeletal and connective tissue disorders [e]					0.293	-
Arm A ($N = 31$)	31 (100)	0 (0.0)	0 (0.0)	0 (0.0)		
Arm B ($N = 28$)	27 (96.4)	1 (3.6)	0 (0.0)	0 (0.0)		
Arm C ($N = 30$)	30 (100)	0 (0.0)	0 (0.0)	0 (0.0)		
Nervous system disorders [f]					0.711	0.091
Arm A ($N = 31$)	16 (51.6)	14 (45.2)	1 (3.2)	0 (0.0)		
Arm B ($N = 28$)	15 (53.6)	13 (46.4)	0 (0.0)	0 (0.0)		
Arm C ($N = 30$)	11 (36.7)	14 (46.7)	5 (16.7)	0 (0.0)		
Vascular disorders [g]					0.293	0.147
Arm A ($N = 31$)	31 (100)	0 (0.0)	0 (0.0)	0 (0.0)		
Arm B ($N = 28$)	27 (96.4)	0 (0.0)	1 (3.6)	0 (0.0)		
Arm C ($N = 30$)	28 (93.3)	0 (0.0)	2 (6.7)	0 (0.0)		

NOTE: Arm A, placebo; arm B, ivermectin 600 μg/kg + placebo for 5 days; and arm C, ivermectin 1200 μg/kg for 5 days.
[a] Includes photophobia, photopsia, blurred vision, visual impairment and vitreous floaters.
[b] Includes abdominal pain, diarrhoea, nausea and vomiting.
[c] Includes fatigue, gait disturbance and malaise.
[d] Includes COVID-19 (coronavirus disease 2019) pneumonia.
[e] Includes arthralgia.
[f] Includes dizziness, headache, paraesthesia and somnolence.
[g] Includes hypotension.



Key	Arm	T0	T4	T48	T72	AUC
768-1	Arm A: placebo	0	0	0		0
768-5	Arm A: placebo	0	0	0		0
776-3	Arm A: placebo	0	0	0		0
776-6	Arm A: placebo	0	0	0		0
776-9	Arm A: placebo	0	0	0		0
776-63	Arm B: I-600	39.9	79.2	22.6		2478
776-1	Arm B: I-600	44.1	140.5	40.9		4360
776-65	Arm B: I-600	71.5	142.6	51.1		4690
776-74	Arm B: I-600	70.9	202.4	70.4		6548
776-4	Arm B: I-600	58.3	305.1	42.3		8370
776-54	Arm B: I-600	169.5	327.3	95.5		10295
776-7	Arm B: I-600	88.6	414.8	89.3		12097
776-2	Arm C: I-1200	161.1	337.1		88.6	13304
768-2	Arm C: I-1200	381.9	483.3	245.3		17760
776-11	Arm C: I-1200	514.3	1082		537.4	50096

Fig. 2. Pharmacokinetic results for ivermectin concentrations in 15 participants (Key). The drug concentration was measured at baseline (T0) and at 4 h (T4), 48 h (T48) and 72 h (T72) after the fifth dose administration (where applicable). The area under the concentration–time curve (AUC) is also reported both graphically and numerically for the 10 participants for whom concentrations were measured.

ranged from 2478 to 12097 for arm B and from 13304 to 50096 for arm C. The decline in viral load at Day 7 and the AEs observed in participants with PK evaluations are listed in Supplementary Table S13. No significant relationship was observed between viral load changes and plasma levels of ivermectin.

4. Discussion

This was the study with the highest dose of ivermectin ever used in a clinical trial for the treatment of COVID-19 or, indeed, for any other condition. The higher dosage (1200 μg/kg for 5 days) showed no safety concerns as no SADRs were observed. The co-primary efficacy outcome was the reduction in viral load on Day 7. The reduction (expressed in $\log_{10}$) was 2.9 for the higher dose (arm C), 2.5 for the lower dose (arm B) and 2.0 for placebo, but the differences were not statistically significant. No significant differences were observed in the clinical outcome either, although the study was not powered sufficiently to detect differences in the secondary outcomes. Concerning AEs, three of the four SAEs (hospitalisation for worsening of the disease) reported were in arm C and 1 in arm B.

Mild or moderate AEs were numerous and reported in all three groups, with the highest proportion in arm C. Of 14 treatment interruptions, 12 were due, precisely, to side effects, of which 11 were in the higher-dose arm (arm C).

The rationale for using a very high dosage was based on the in vitro study published by Caly et al. [8]. The foreseeable plasma concentration after the fifth day of treatment made effective drug levels in pneumocytes plausible, also considering that much higher levels are likely to be achieved in pneumocytes than in plasma [21]. The dosages used in our study were selected according to the PK considerations reported in the study protocol and in the Introduction: the C_{max} after the fifth dose should be of the same order as after a single dose of 2 mg/kg, already found to be safe in healthy volunteers [11]. Our PK results, albeit on a limited number of participants, confirmed our initial hypotheses, indeed showing slightly higher concentrations than foreseen, and reaffirm the biological plausibility of high-dose ivermectin for COVID-19. However, our study failed to show a significant effect on viral load decline.

This study confirms that ivermectin at high dose can be considered safe. However, a high proportion of dropouts, albeit for mild or moderate side effects, was observed in the higher-dose treatment arm. It is true that symptomatic subjects at greater risk of illness progression might be more motivated to continue a treatment in the presence of minor side effects. However, other considerations make a new trial on high-dose ivermectin problematic. First of all, no sign of a possible positive clinical outcome was highlighted. On the contrary, the four participants whose disease worsened enough to cause hospitalisation were all in the treatment arms, and three of these were in the higher-dose arm. Although this is an observation, rather than a statistically valid conclusion, it might be questioned whether ivermectin itself could have at least partly contributed to the clinical worsening. Indeed, there are concerns due to its mechanism of action, which is not directly against the virus but implies inhibition of a host protein involved in intracellular transport [22]. However, the clinical features of the hospitalised participants here were deemed compatible with COVID-19 evolution, and no major neurological signs were observed, as reported previously in cases of serious ivermectin toxicity [23].

Self-administration of veterinary formulations of ivermectin has been widely reported since the initial (and weak) evidence about its possible efficacy against COVID-19. Toxic effects (mostly gastrointestinal distress and neurological symptoms) have been reported following such misuse [24], and the World Health Organization (WHO) has recommended to limit the administration of ivermectin for COVID-19 only for clinical trials [25].

Different groups have carried out systematic reviews with meta-analysis on the use of ivermectin for COVID-19 [26–29]. Results between different works are inconsistent, and the quality of clinical trials included in some reviews has been questioned. On one hand, fraudulent data have been detected; on the other, the sample size of many trials was too small or the study quality was assessed as low [28,30]. Amongst other reviews, a Cochrane meta-analysis has been published [28]. It analysed 14 randomised trials with ivermectin for COVID-19 published until 26 May 2021 including a total of 1678 participants, finding no evidence favouring ivermectin for clinical outcomes or for viral clearance. In all studies, the dosage used was much lower than that of our lower dosage arm, with the exception of the study by Krolewiecki et al. in Argentina [31] that used the same dose as our lower-dose arm (600 μg/kg). The latter study showed a reduction in viral load in treated subjects versus controls in a subgroup of participants in whom the plasma concentration of the drug, measured 4 h after dosing, exceeded 160 ng/mL.

Our study has some limitations. The first is that it failed to reach the planned sample size. However, the conditional power (CP) analysis showed that even reaching the target sample size, the hypothesised effect would hardly be demonstrated (arm B vs. arm A, CP = 0.001; arm C vs. arm A, CP = 0.27). Another limitation was the extreme difficulty in recruiting participants. Approximately 90% of subjects screened were not eligible to be included for various reasons, including a high proportion of refusal to give their consent. Moreover, 79 (84.9%) of the 93 study participants were recruited by the co-ordinating site. Major strengths of this study were the double-blind design, the inclusion of a placebo arm and the lack of any other major deviations from the study protocol.

In conclusion, we did not demonstrate a significant reduction in viral load between ivermectin and placebo, although a trend for the highest dose is apparent. Whether this drug might have clinical efficacy at lower doses remains debated. We believe that our findings further support the WHO recommendation [25] suggesting that it is currently advisable to refrain from administrating ivermectin for the treatment of COVID-19 outside of clinical trials. Considering the reduced tolerability, large, high-dose clinical trials should not be recommended.

Contributors

A complete list of investigators/contributors is reported in the Supplementary material.

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Competing interests

None declared.

Ethical approval

The protocol received clearance from the Ethics Committee of INMI–Spallanzani in Rome, which is competent for all COVID-19 trials in Italy [resolution 139/2020 of 28 May 2020].

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2021.106516.

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