SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori"
Istituto di Ricovero e Cura a Carattere Scientifico

ISTITUTO ROMAGNOLO PER LO STUDIO DEI TUMORI DINO AMADORI

PROTECT: A randomized study with Hydroxychloroquine versus observational support for prevention or early phase treatment of Coronavirus disease (COVID-19)

Protocol Code: IRST100.47
IRST Identifier Code: L2P2266
Eudract number: 2020-001501-24

CLINICAL STUDY REPORT

30/09/2022

Study Activated:	28/04/2020
First Patient Enrolled:	14/05/2020
Anticipated Accrual:	2300 subjects
Actual Accrual:	156 subjects

Study Chair:
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Outline of Report

SUMMARY OF STUDY PROTOCOL

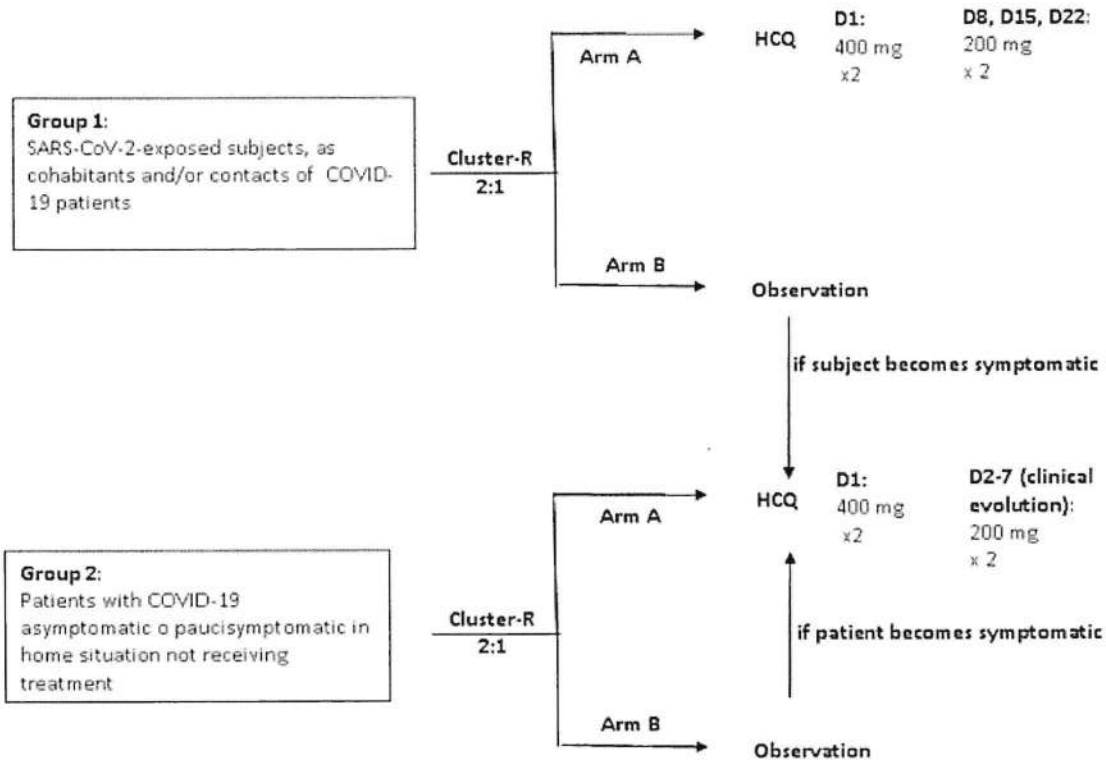
Project Title:	PROTECT: A randomized study with Hydroxychloroquine versus observational support for prevention or early phase treatment of Coronavirus disease (COVID-19)
Study Design:	This is a Italian, superiority, open label cluster-randomised, interventional clinical trial aimed at assessing whether the treatment with Hydroxychloroquine can reduce the percentage of symptomatic subjects compared to observation only in household members/contacts of COVID-19 patients (Group 1) and if the treatment with Hydroxychloroquine could be introduced in early phase COVID-19 population (Group 2). The participants will be randomised to receive either: Arm A) hydroxychloroquine vs Arm B) Observation (2:1 randomisation).
Background and rationale:	Novel pneumonia caused by a previously unknown pathogen emerged in Wuhan. The pathogen was soon identified as a novel coronavirus (2019-nCoV), which is closely related to severe acute respiratory syndrome CoV. Currently, there is neither specific treatment nor prophylaxis against the new virus SARS-CoV-2 in healthy subjects. Therefore, the current emergency situation warrants for the urgent development of potential strategies to protect people at high risk of infection, particularly cohabitants of diagnosed COVID-19 patients. Antiviral drugs administered shortly after symptom onset can reduce the spread of infection by reducing viral shedding in the respiratory secretions of patients (SARS-CoV-2 viral load in sputum peaks at around 5-6 days after symptom onset and lasts up to 14 days), and targeted prophylactic treatment of contacts could reduce their risk of becoming infected. Implementing antiviral treatment (particularly for COVID19 patients, untreated, and only observed during at-home quarantine) and prophylaxis requires adequate drug availability, and the safety of the procedure must be high. Hydroxychloroquine is a drug that is available for chemoprophylaxis and treatment of malaria. It is registered and used as a disease-modifying antirheumatic drug. It has a long history, is safe and well-tolerated at typical doses. Moreover, hydroxychloroquine show to have antiviral activity in vitro against coronaviruses and specifically SARS-CoV-2. Hydroxychloroquine may be a promising drug for the prevention and the cure of SARS-CoV-19. Ingested days before the virus is introduced to the body, it will reach the serum concentration ranging the EC50 values of 6.25 and 5.85 micromolar at 24 and 48 hours. The drug can accumulate at high levels in lung tissue. Based on physiological pharmacokinetic models studies and by in vitro data results, the possibility to reach high concentrations of hydroxychloroquine in lung fluid was demonstrated. A single dose of hydroxychloroquine at 800 mg may provide a lung tissue concentration that is more than twenty times higher than EC50 values necessary to inhibit SARS-CoV-2 in the lung on day 1. It is plausible that a single dose of 400 mg or even 200 mg can provide adequate lung tissue concentration to inhibit SAR-CoV-2. Since the half-life after a single dose of 200 mg is 22 days, a single dose every three weeks should be sufficient for the prevention of SARS-CoV-2 induced lung damage. The blood or sinus concentrations may

	not be enough to eradicate the virus; however, prevention of lung damage may convert this deadly infection into an upper respiratory infection. Several drugs, such as chloroquine have been used in patients with SARS or MERS. Standard assays were carried out to measure the effects of this compound on the cytotoxicity, virus yield and infection rates of 2019-nCoVs. Chloroquine blocked virus infection at low-micromolar concentration and showed high selectivity index. Chloroquine and Hydroxychloroquine is known to block virus infection by increasing endosomal pH required for virus cell fusion and glycosylation of viral surface proteins. Besides its antiviral activity, chloroquine has an immune-modulating activity, which may synergistically enhance its antiviral effect in vivo. Several clinical trials with Hydroxychloroquine treatment for COVID-19 are ongoing in China (NCT04261517 and NCT0437693). From the first study preliminary data are available, but they are not conclusive because of the small sample size. As the COVID-19 spreads, efforts are made to reduce transmission via standard public health interventions based on isolation of cases and tracing contacts, but such strategy could contribute to reducing the overall size of an outbreak, but will still not be sufficient to achieve outbreak control of COVID-19 when the basic reproduction number (R0) is higher than 1.5 or the proportion of contacts traced is lower than 80%. Another assumption is that isolation of cases is 100% effective in stopping transmission, yet home confinement of infected individuals and contacts is challenging, efficacy is variable, and rigorous tracking involves a considerable amount of public health resources. Therefore, our institute is planning a controlled cluster-randomised study with Hydroxychloroquine versus observation for prevention of Coronavirus disease (COVID-19) in SARS-CoV-2-exposed subjects with an intermediate-high risk of infection and for treatment of early phase COVID-19 patients.	
Study Population:	**Group 1:** SARS-CoV-2-exposed subjects, as household members/contacts of COVID-19 patients. **Group 2:** Patients with COVID-19 asymptomatic o paucisymptomatic in home situation.	
Objectives:	**Objectives**	**Outcomes**
Primary Objectives:	**Group 1:** Prevention of COVID-19 or related symptoms in household members/contacts of COVID-19 patients within one month from randomization **Group 2:** Efficacy of Hydroxychloroquine in early phase COVID-19 within 14 days from randomization	**Group 1:** The primary endpoint/outcome measure is the proportion of subjects of Group 1 who become symptomatic and/or swab positive in each arm within 1 month from randomization. **Group 2:** The primary endpoint/outcome measure is the proportion of subjects of Group 2 who become swab negative in each arm within 14 days from randomization.

Main Secondary Objectives	1. To compare the efficacy of prophylaxis with Hydroxychloroquine in prevention of COVID-19 infection (swab positive) in a population of SARS-CoV-2-exposed subjects composed by household members/contacts of COVID-19 patients respect to observation only.	1. The proportion of subjects with positive swab in randomized population of SARS-CoV-2-exposed subjects (Group 1) within 1 month from randomization in both arms.
	2. To assess the efficacy of prophylaxis with Hydroxychloroquine in subgroup population identified by stratification factors, class of age and gender.	2. The proportion of subjects of Group 1 who become symptomatic in each arm within 1 month from randomization, in subgroup population identified by stratification factors, class of age and gender.
	3. To assess the efficacy of Hydroxychloroquine in early phase COVID-19 patients within 14 days from randomization in subgroup population identified by stratification factors, class of age and gender	3. The proportion of subjects of Group 2 who become swab negative in each arm within 14 days from randomization, in subgroup population identified by stratification factors, class of age and gender.
	4. To assess the efficacy of Hydroxychloroquine in early phase COVID-19 patients within 1 month from randomization in overall population and in subgroup population identified by stratification factors, class of age and gender.	4. The proportion of subjects of Group 2 who become swab negative in each arm within 1 month from randomization in overall population and in subgroup population identified by stratification factors, class of age and gender.
	5. To evaluate, treatment toxicity of hydroxychloroquine in SARS-CoV-2-exposed subjects population and COVID-19 patients	5. Absolute and relative frequencies of Serious Adverse Events (CTCAE version 5.0) in both arms for the Group 1 and Group 2.
	6. To evaluate Quality of Life (EQ-5D-5L) from SARS-CoV-2-exposed subjects population and COVID-19 patients	6. Variation in Quality of Life scores in different time points (weekly) respect to baseline values in both Group 1 and Group 2 populations.
Main Inclusion Criteria:	Inclusion criteria: 1. Male or Female, aged >=18 years 2. SARS-CoV-2-exposed subjects, as households and/or contacts of COVID-19	

	patients (Group 1). In this group are included health care professionals in contact with COVID19 patients. or 3. Patients with COVID-19, asymptomatic or paucisymptomatic in home situation who are not in treatment with any anti COVID-19 medication (Group 2) 4. Absence of any COVID-19 symptom in last week before randomization (fever >37.5°C, cough, dyspnea) (only for group 1 subjects) 5. Paracetamol treatment is accepted only for group 2. 6. Participant is willing and able to give informed consent for participation in the study (either recorded during a telephonic interview or signed in person) and agrees with the study and its conduct.
Study treatment:	The participants will be cluster-randomised (2:1 randomisation) to receive either: **Arm A) Hydroxychloroquine** **Group1:** A loading dose Hydroxychloroquine 400 mg twice daily at day 1, followed by a weekly dose of Hydroxychloroquine 200 mg twice daily on days 8, 15 and 22, covering a total of 1 month of treatment. **Group 2:** A loading dose Hydroxychloroquine 400 mg twice daily at day 1 followed by 200 mg twice daily for a total of at least 5-7 days according to clinical evolution (Region of Emilia Romagna Guideline). **Arm B) Observation.**
Sample Size:	**For Group 1**: A sample size of about 2000 SARS-CoV-2-exposed subjects as household members and/or contacts of COVID-19 patients will participate into the study. Assuming about 1.5-2.0 asymptomatic household members and/or contacts for each COVID-19 patient, we expected to identify approximately 1000-1300 COVID-19 index cases. **For Group 2:** Sufficient power for primary objective (negative swab within 14 days from randomization) will be reached, given a sample size of 300 COVID-19 subjects asymptomatic or paucisymptomatic in home situation not treated for COVID-19 (25-30% of about 1000- 1300 expected case index COVID-19 patients). Since up to date reduced evidences about COVID-19 infection epidemiology, the continuous update of diagnostic and therapeutic approaches, the sample size estimation could be updated after a one third of population will be recruited and eventually modified according a substantial protocol amendment
Statistical analysis:	We planned a Generalized Estimating Equation analysis to get advantage of subject-specific covariates. This is therefore more efficient than a cluster level analysis. The above reported sample size analysis is therefore to be considered conservative.

STUDY SCHEMA



All Households members and/or contacts fulfilling all inclusion criteria (for Group 1) of each COVID-19 patient, will be enumerated into a single cluster (information of each subject will be recorded in specific data record) and these clusters will be cluster-randomised (2:1) to either arm A or arm B.
Randomization lists will be stratified according to the following factors:
- COVID-19 risk level for residence (high vs low/intermediate);
- Health care professionals (yes vs no)
- Home situation without COVID-19 treatment (yes vs no)

COVID-19 index cases will be randomized (2:1) to either arm A or arm B.
An independent statistician not otherwise involved in the trial will generate the allocation sequence, and COVID-19 response teams will be unaware of the allocation of clusters.

Treatment Schedule:

Arm A) Hydroxychloroquine

Group1:
A loading dose Hydroxychloroquine 400 mg twice daily at day 1, followed by a weekly dose of Hydroxychloroquine 200 mg twice daily on days 8, 15 and 22, covering a total of 1 month of treatment.

Group 2:
A loading dose Hydroxychloroquine 400 mg twice daily at day 1 followed by 200 mg twice daily for a total of at least 5-7 days according to clinical evolution.

Arm B) Observation

Amendment/Notification Summary:

The protocol has been amended (Amendment 1.0 of 05/08/2020) to :

- Prolong the study duration in view of the evolution of the epidemic in Italy.
- Update the background with literature data concerning the use of hydroxychloroquine in the treatment and prevention of COVID-19 and announcements by regulatory bodies (EMA, AIFA) suspending the use of hydroxychloroquine outside clinical trials.
- add the evaluation of the clinical evolution of COVID-19 in Group 2 patients treated and not treated with hydroxychloroquine as a secondary objective.
- Update the eligibility criteria in accordance with the recommendations of the Ethics Committee (in particular, the exclusion criterion regarding HIV, HCV and HBV positivity has been eliminated
- Update the study procedures regarding informed consent process and the timing of swabs according current clinical practice
- Update the list of Public Health Departments collaborating to the project.

1. INTRODUCTION

This is an open label, superiority, cluster-randomized Italian interventional clinical trial, evaluating the role of Hydroxychloroquine versus observation only in preventing infection to COVID-19 or treating early phase COVID-19 patients.
The Cluster-randomization, around index case, was considered the most appropriate design, as the lack of independence among participants could not be excluded. In this study the index case is a person newly diagnosed with COVID-19 and relative cluster is composed by SARS-CoV-2-exposed subjects, as household members and contacts of the COVID-19 patient.
Each index case has been randomised to either Arm A: Hydroxychloroquine or Arm B: observation in a 2:1 ratio on an open label basis. Participants in the same cluster received the same intervention.
All participating subjects becoming paucisymptomatic/symptomatic could continue/start, out of random, hydroxychloroquine, if not otherwise treated by treating physician or if not contraindicated.

Study population was constituted by:
Group 1: SARS-CoV-2-exposed subjects, as household members/contacts of COVID-19 patients.
Group 2: Patients with COVID-19 asymptomatic or pauci-symptomatic without specific COVID-19 treatment.

For Group 1:
A sample size of about 2000 SARS-CoV-2-exposed subjects, as household members/contacts of COVID-19 patients were planned to be enrolled into the study. Assuming about 1.5-2.0 asymptomatic household members/contacts for each COVID-19 patient, we expected to identify approximately 1000-1300 COVID-19 index cases.
For Group 2:
Sufficient power for primary objective (negative swab within 14 days from randomization) would have been reached, given a sample size of 300 COVID-19 subjects asymptomatic or paucisymptomatic in home situation not treated for COVID-19 (25-30% of about 1000-1300 expected case index COVID-19 patients).

The sample size estimation was planned to be updated after a one third of the population would have been recruited and eventually modified according a substantial protocol amendment. -The overall study duration would have been 16 months; 12 months for subjects enrollment, and 1 month of treatment and further 3 months of follow-up

The primary endpoint of the study was:
For Group 1: the proportion of subjects of Group 1 who become symptomatic and/or swab positive in each arm within 1 month from randomization.
For Group 2: the proportion of subjects of Group 2 who become swab negative in each arm within 14 days from randomization.
Secondary endpoints were:
1. The proportion of subjects with positive swabs in randomized population of SARS-CoV-2-exposed subjects (Group 1) within 1 month from randomization in both arms.
2. The proportion of subjects of Group 1 who become symptomatic in each arm within 1 month from randomization, in subgroup population identified by stratification factors, class of age and gender.
3. The proportion of subjects of Group 2 who become swab negative in each arm within 14 days from randomization, in subgroup population identified by stratification factors, class of age and gender.

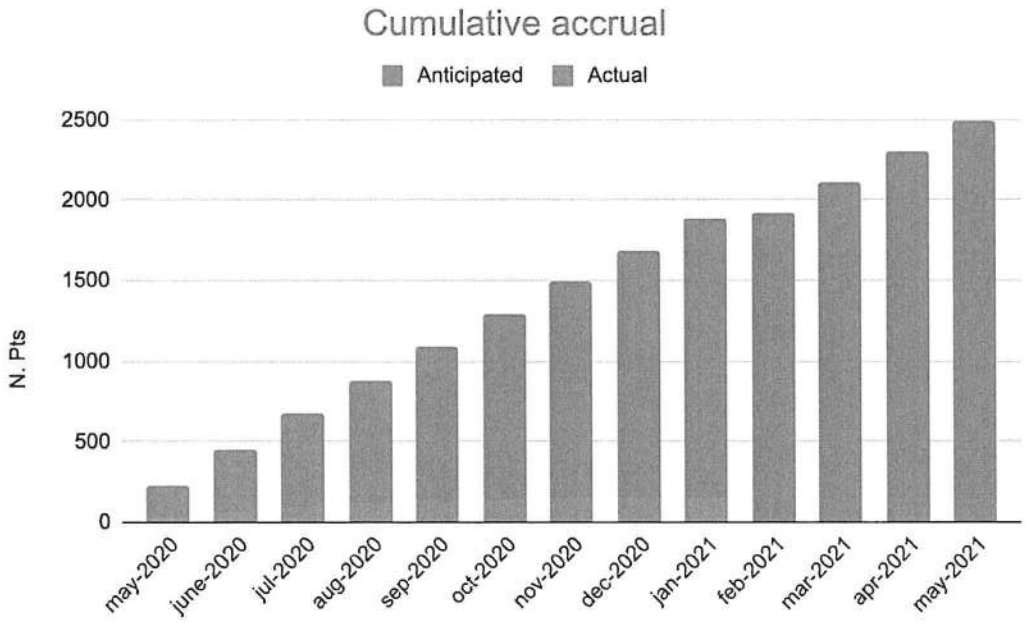
4. The proportion of subjects of Group 2 who become swab negative in each arm within 1 month from randomization in overall population and in subgroup population identified by stratification factors, class of age and gender.
5. Absolute and relative frequencies of Serious Adverse Events (CTCAE version 5.0) in both arms for the Group 1 and Group 2.
6. Variation in Quality of Life scores in different time points (weekly) respect to baseline values in both Group 1 and Group 2 populations.
7. To evaluate the clinical evolution and outcome of COVID-19 disease in Group 2 population

2. ACCRUAL

The study was approved by AIFA (Italian Medicines Agency) and the Ethics Committee of the IRCCS Italian Experimental Institute "Lazzaro Spallanzani" (Rome) - as the competent ethic committee for all clinical trials conducted in Italy on COVID-19 - on 28/04/2020 and the first subject was enrolled on 14/05/2020 (last patient was enrolled on 15/01/2021).
Eight public health departments have been involved in the project (Forlì, Ravenna, Rimini, Bologna, Ferrara, Parma, Reggio Emilia, Alessandria) for the notification of new cases and contact.
A total of 181 subjects were contacted and screened for eligibility over a period of 8 months; of these, 19 patients did not meet eligibility criteria, 4 patients did not consent to participate, 2 patients withdrew consent before randomization.
A total of 156 subjects were randomized: 77 in group 1 (52 in arm A and 25 in arm B) and 79 in group 2 (53 in arm A and 26 in arm B).

Figure 1 shows the cumulative anticipated and actual accrual. The study was terminated prematurely on 26/01/2021 due lack of enrollment.

Figure 1 - Cumulative accrual



3. PATIENT CHARACTERISTICS

Table 3.1 gives the characteristics of evaluable patients.

Table 3.1 Randomized patients with evaluable data according to treatment

	GROUP 2 (COVID-19)		GROUP 1 (CONTACTS)	
	Arm A: HCQ (n=53)	Arm B: Observation (n=26)	Arm A: HCQ (n=52)	Arm B: Observation (n=25)
	N. (%)	N. (%)	N. (%)	N. (%)
Age (years)				
18-64	51 (96.2)	25 (91.2)	50 (96.2)	20 (80.0)
65-84	2 (3.8)	1 (3.8)	2 (3.8)	5 (20.0)
≥85	0	0	0	0
Median (range, IQR)	44 (19-69)	44 (18-70)	41 (18-72)	51 (18-75)
Mean value (SD)	41.2 (12.1)	43.2 (14.4)	41.4 (14.7)	50.7 (16.1)
Gender				
Male	28 (53.8)	19 (73.1)	30 (57.7)	14 (56.0)
Female	24 (46.2)	7 (26.9)	22 (42.3)	11 (44.0)
Ethnic origin				
European	37 (69.8)	18 (69.2)	47 (92.2)	24 (96.0)
Asian	8 (15.1)	4 (15.4)	2 (3.9)	0
African	6 (11.3)	4 (15.4)	2 (3.9)	1 (4.0)
Other	2 (3.8)	0	0	0
Unknown/missing	0	0	1	0
Blood type				
0+	13 (24.5)	2 (7.7)	18 (34.6)	3 (12.0)
0-	1 (1.9)	0	4 (7.7)	6 (24.0)
A+	8 (15.1)	4 (15.4)	6 (11.5)	3 (12.0)
A-	2 (3.8)	0	1 (1.9)	0
B+	7 (13.2)	3 (11.5)	2 (3.9)	1 (4.0)
AB+	1 (1.9)	0	0	0
Unknown/missing	21 (39.6)	17 (65.4)	21 (40.4)	12 (48.0)
Province of residence				
AL	0	0	1 (1.9)	0
PR	1 (1.9)	0	0	0
RE	3 (5.7)	3 (11.5)	4 (7.7)	5 (20.0)
BO	0	0	12 (23.1)	5 (20.0)
FE	3 (5.7)	0	3 (5.8)	3 (12.0)
RA	38 (71.6)	18 (69.3)	17 (32.7)	11 (44.0)
FC	7 (13.2)	5 (19.2)	15 (28.8)	1 (4.0)
RN	1 (1.9)	0	0	0
Risk level for residence (index cases)				
High	3 (5.7)	3 (11.5)	5 (9.6)	5 (20.0)
Intermediate/Low	50 (94.3)	23 (88.5)	47 (90.4)	20 (80.0)
Health care professional (index cases)				
No	45 (84.9)	22 (84.6)	41 (78.8)	21 (84.0)
Yes	8 (15.1)	4 (15.4)	11 (21.2)	4 (16.0)
Medical doctor	1	1	2	1
Nurse	3	0	2	2
Other	3	2	6	1
Unknown/missing	1	1	1	0
Department where the health care				

professional works				
Emergency	0	0	0	0
Resuscitation	1	0	0	0
Infectious diseases	1	0	2	1
Other	5	3	8	3
Unknown/missing	1	1	1	0

HCQ, Hydroxychloroquine

Group 2 subjects had a median age of 44 years (range 18-70), 60% were male and 70% were of European ethnic origin. 90% of them were from a low/intermediate risk level for residence of contracting COVID-19 and 15% were health care professional (nurse/other).

Table 3.2 summarizes the symptoms present at the diagnosis of COVID-19 with their intensities. As can be seen, most of the subjects enrolled had loss of taste/smell, sore throat and/or cold, muscle/bone pain or pain to the sternum.

Table 3.2 Symptoms intensity at the time of diagnosis of COVID-19

	GROUP 2 (COVID-19)	
	Arm A:HCQ (n=53)	**Arm B: Observation (n=26)**
Symptoms intensity	**N. (%)**	**N. (%)**
Loss of taste/smell		
No	36 (67.9)	18 (69.2)
Mild	9 (17.0)	5 (19.2)
Moderate	6 (11.3)	0
Severe	2 (3.8)	3 (11.6)
Sore throat and/or cold		
No	32 (60.4)	16 (61.6)
Mild	18 (34.0)	8 (30.8)
Moderate	3 (5.6)	1 (3.8)
Severe	0	1 (3.8)
Muscle/bone pain		
No	40 (75.5)	19 (73.1)
Mild	10 (18.9)	5 (19.2)
Moderate	3 (5.6)	2 (7.7)
Severe	0	0
Pain to the sternum		
No	46 (86.8)	26 (100)
Mild	6 (11.3)	0
Moderate	1 (1.9)	0
Severe		
Nausea		
No	49 (92.4)	25 (96.2)
Mild	3 (5.7)	1 (3.8)
Moderate	1 (1.9)	0
Severe	0	0
Diarrhea		
No	50 (94.3)	25 (96.2)
Mild	2 (3.8)	1 (3.8)
Moderate	1 (1.9)	0
Severe	0	0
Cough		
No	36 (67.9)	20 (76.9)

Mild	17 (32.1)	4 (15.4)
Moderate	0	2 (7.7)
Severe	0	0
Shortness of breath		
No	49 (92.5)	24 (92.3)
Mild	4 (7.5)	2 (7.7)
Moderate	0	0
Severe	0	0
Conjunctivitis		
No	52 (98.1)	25 (96.2)
Mild	1 (1.9)	1 (3.8)
Moderate	0	0
Severe	0	0
Vomiting		
No	53 (100)	26 (100)
Mild	0	0
Moderate	0	0
Severe	0	0

With reference to the subjects of group 2 (COVID-19), the diagnosis of COVID-19 was mainly made by nasal swab (75% in arm A and 69% in arm B) and home isolation was necessary for about 90% of the subjects enrolled while the remaining 10% underwent COVID-19 hotel isolation (Table 3.3).
As for the subjects of group 1 (observation), 73% of the subjects were cohabiting with the COVID-19 subject; the latter had no symptoms in 45% of cases, while 30-36% had had symptoms for less than a week.

Table 3.3 Additional information present at the time of diagnosis of COVID19

	GROUP 2 (COVID-19)		GROUP 1 (CONTACTS)	
	Arm A: HCQ (n=53)	Arm B: Observation (n=26)	Arm A: HCQ (n=52)	Arm B: Observation (n=25)
	N. (%)	N. (%)	N. (%)	N. (%)
Swab for COVID-19 carried out in the last week of diagnosis				
No	0	0	17 (32.7)	12 (48.0)
Yes	53 (100)	26 (100)	35 (67.3)	13 (52.0)
only nasal	40 (75.5)	18 (69.2)	0	0
only oropharyngeal	5 (9.4)	4 (15.4)	0	0
nasal and orpharyngeal	8 (15.1)	4 (15.4)	0	0
In isolation (only for group 2)				
No	0	0	-	-
No, hospitalized	0	0	-	-
Yes, home care	49 (92.4)	23 (88.5)	-	-
Yes, COVID Hotel	4 (7.6)	3 (11.5)	-	-
Unknown/missing	0	0	-	-
Only for Group 1:				
Relationship with the subject COVID-19				
Cohabitant	-	-	37 (72.5)	18 (75.0)
Business colleague	-	-	1 (2.0)	2 (8.3)
Other	-	-	13 (25.5)	4 (16.7)
Unknown/missing	-	-	1	1
Did the subject COVID-19 you came into contact with have				

symptoms?				
No	-	-	15 (45.4)	5 (45.5)
Yes, less than a week from now	-	-	10 (30.3)	4 (36.4)
Yes, for more than a week and less than 2 weeks from now	-	-	6 (18.2)	0
Yes, for two weeks and less than a month from now	-	-	0	2 (18.2)
Yes, for more than a month from now	-	-	1 (3.0)	0
I do not know	-	-	1 (3.0)	0
Unknown/missing	-	-	19	14

4. TREATMENT SUMMARY

Table 4.1 summarizes the compliance with the treatment by arm. 87% of the subjects in group 2 regularly took hydroxychloroquine while in group 1 the adherence to treatment was 81%.

Table 4.1 Compliance with the treatment

	GROUP 2 (COVID-19)	GROUP 1 (CONTACT)
	Arm A: HCQ (n=53)	Arm A: HCQ (n=52)
	N. (%)	N. (%)
Regular intake of HCQ		
Yes	46 (86.8)	42 (80.8)
No	7 (13.2)	10 (19.2)
Refusal	5[1]	7[2]
Forgetfullness	1	1
Investigator's decision	1	2
Unacceptable toxicity	0	0
Other	0	0

[1]2 patients never started treatment (PROTECT_N0143_I_0001 and PROTECT_N0150_I_0001)
[2]2 patients never started treatment (PROTECT_N0075_C_0001 and PROTECT_N0199_C_0002)

5. OUTCOMES

5.1 Primary objective

The percentage of **Group 1** subjects who become symptomatic and/or positive within 1 month of randomization is 2.6% (1.9% in the hydroxychloroquine arm and 4% in the control arm), with a median time of 8 days in the hydroxychloroquine arm and 5 days in the control arm.

The percentage of **Group 2** subjects who become swab negative in each arm within 14 days of randomization was 88.6% of the subjects included in the study (76.5% in the control arm and 96.3% in the hydroxychloroquine). The median time between the date of randomization and the date of execution of the next swab was 12 days for the control arm and 9.5 days for the hydroxychloroquine arm.

5.2 Secondary objectives:

SAFETY

During the study period, no safety results emerged that made it necessary to take any action regarding the conduct of the clinical trial covered by this report.
The protocol and the investigator brochure was modified to include new safety warnings that have become available. In particular, following the publication of some observational studies reporting an increased risk of serious heart disease including arrhythmias and cardiac arrest, the COVID-19 EMA Pandemic Task Force (COVID-ETF) issued a statement to draw attention of health professionals on the risks of serious side effects in the case of the use of chloroquine or hydroxychloroquine in patients with COVID-19, especially considering the higher dosages used.
Hydroxychloroquine, with AIFA decision of 17/03/2020, was included in the list of drugs that could be used for the "treatment of patients affected by SARS-CoV2 (COVID-19) infection", also in the home regime, for 3 months (off label use).
On 29/05/2020, AIFA recommended using chloroquine and hydroxychloroquine only in clinical trials for the treatment or prophylaxis of COVID-19 under strict control.
No new risks were identified in the context of this study.
The incidence of non-serious adverse events was consistent with the expected adverse event profile of the product. No serious adverse events have been observed.
The overall risk/benefit balance of the product used in the study program remained unchanged and positive.
No major new risks were identified in the context of this study. All evaluable patients were considered for safety evaluation. **Table 5.1** summarizes the symptoms intensity and/or AEs reported separated by the two treatment arms and groups.

Table 5.1. Symptoms intensity and/or adverse events encountered during treatment

	Group 2 (COVID-19)		Group 1 (CONTACT)	
	Arm A: HCQ (n=53)	Arm B: Observation (n=26)	Arm A: HCQ (n=52)	Arm B: Observation (n=25)
	N.	N.	N.	N.
Shortness of breath				
Mild	4	1		
Moderate	0	1		
Severe				
Cough				
Mild	12	4	3	1
Moderate	3	1		
Severe	1	1		
Loss of taste/smell				
Mild	5	4	1	1
Moderate	3	1	0	1
Severe	5	3		
Sore throat and/or cold				
Mild	11	6	5	3
Moderate	4	1	2	0
Severe				
Pain to the sternum				
Mild	7	0		
Moderate	4	0		
Severe	1	0		
Muscle/bone pain				
Mild				
Moderate				
Severe				
Headache/dizziness				
Mild	14	9	13	3
Moderate	8	0	1	0
Severe				
Diarrhea				
Mild				
Moderate				
Severe				
Nausea				
Mild	7	1	5	
Moderate	3	0		
Severe				
Vomiting				
Mild				
Moderate	1			
Severe				
Abdominal pain				
Mild	7		3	2
Moderate	4			
Severe				
Mood instability				
Mild	8	3	5	2
Moderate	2	0	1	1
Severe				
Heart disorders				

Mild	1	1	3	
Moderate	1			
Severe				
Conjunctivitis				
Mild	2		1	
Moderate		1		
Severe				
Visual disturbances				
Mild	2	1	1	1
Moderate	1	0		
Severe				
Skin disorders				
Mild	7	0	2	0
Moderate	0	2		
Severe				
Allergic reaction				
Mild	4	0	1	0
Moderate				
Severe				
Other				
Mild				
Moderate				
Severe				

6. CONCLUSIONS

The PROTECT study was designed in the very early phase of VOCID-19 pandemic to address the urgent medical need of finding new drugs – or repurposing existing ones – to treat COVID-19 especially in the early phase of disease.

The PROTECT trial was designed following the new requirements regarding CODIV-19 containment. In all phases of the trial, urgency was a driving force. Great efforts were undertaken to set up a properly designed randomized trial in a very short time and with the many critical aspects to afford in the early 'COVID-19 era'. The set-up benefited from a positive exchange with AIFA and, according to the Article 17 of the Law Decree No. 18 of 17th March 2020, the trial protocol has been preliminarily evaluated by the AIFA Technical Scientific Committee (CTS) and subsequently approved, after evaluation by the AIFA Competent Authority (Clinical Trial Office) and by the Ethics Committee of the National Institute for Infectious Diseases "Lazzaro Spallanzani", as single national Ethics Committee.

The trial was run in a difficult and rapidly-changing context; several factors negatively impacted on the conduction of the PROTECT trial, including the drop down of infected people that has been observed after the first lockdown period and the concerns about Hydroxychloroquine safety and efficacy following the publication of some trials reporting lack of efficacy and some observational studies reporting cardiac toxicity. The resonance that this news had in the press and media contributed to a climate of low confidence on behalf of the subjects contacted.

As a consequence, the study has been prematurely stopped due to insufficient recruitment. Therefore, concerning the primary endpoint of efficacy, it was not possible to obtain any statistically significant result and to draw any conclusion.

As far as safety is concerned, no serious adverse events have been observed in our trials, and the incidence of non-serious adverse events was consistent with the expected adverse event profile of the product.

7. PUBLICATIONS

A first manuscript, relating to the study protocol, was published by our group in the international scientific journal Trials: Nanni O et al. PROTECT Trial: A cluster-randomized study with hydroxychloroquine versus observational support for prevention or early-phase treatment of Coronavirus disease (COVID-19): A structured summary of a study protocol for a randomized controlled trial. Trials 2020 Jul 31; 21 (1): 689. doi: 10.1186 / s13063-020-04527-4, to give visibility to the project and enlarge the recruitment possibilities.

A second manuscript, concerning the feasibility of conducting the study, was also published by our group in the international scientific journal Epidemiology and Prevention: Lilli C et al. Is it possible to conduct clinical trials during a pandemic? The example of a trial of hydroxychloroquine. Epidemiol Prev 2021 Jan-Apr; 45 (1-2): 28-36. doi: 10.19191 / EP21.1-2.P028.036.