TITLE PAGE

Investigational product: FDA-135 (Bromhexine)

SARS-CoV-2 infection

Sponsor: Agencia Estatal Consejo Superior de Investigaciones Científicas, M.P (CSIC)

Protocol Title:

A Randomized, Open-label, Standard-of-Care Comparative, Drug Repositioning Clinical Trial to Evaluate the Efficacy and Safety of FDA-135 in Combination with Standard of Care in the Treatment of Infection Caused by SARS-CoV-2, in Patients With early Stage COVID-19 Disease, Receiving Primary Care Treatment.

Design: Phase 3 drug repositioning, parallel, open-label, randomised, comparison with standard of care, 28 days of follow-up, 30 ml daily of Bromhexine during 7 days of treatment in patients with early stage COVID-19 disease (mild to moderate) at Primary Health Care.

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Confidentiality Statement

The information contained in this document, especially unpublished data, is the property of the sponsor of this study, CSIC. Therefore, these are being confidentially provided to you in your capacity as investigator, potential investigator, or consultant for review by you, your staff, and the Ethics Committee for Drug Research. It is understood that this information will not be disclosed to a third party without the written authorization of CSIC., except to the extent necessary to obtain informed consent from persons to whom the medicinal product may be administered.

Ethical Statement

The study was performed in compliance with Good Clinical Practices (GCP), including the archiving of essential documents.

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1. SYNOPSIS

Name of Sponsor/Company: Agencia Estatal Consejo Superior de Investigaciones Científicas, M.P. (CSIC)	Individual Study Table Referring to Part of the Dossier	*(For National Authority Use only)*
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Title of Study:

A Randomized, Open-label, Standard-of-Care Comparative, Drug Repositioning Clinical Trial to Evaluate the Efficacy and Safety of FDA-135 in Combination with Standard of Care in the Treatment of Infection Caused by SARS-CoV-2, in Patients With early Stage COVID-19 Disease, Receiving Primary Care Treatment. Code: CSIC-FDA135-2021-01.

Investigators:

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Study centre(s):

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Publication (reference)

Studied period: (date of first enrolment/date of last completed) February 24th, 2022 /July 28th, 2022	Phase of development: Phase 3

Objectives:

Primary objective: The primary objective is to evaluate the efficacy of Bromhexine combined with standard of care (SOC) on reducing the SARS-CoV-24 viral load from baseline.

Secondary Objective: To evaluate the laboratory efficacy of Bromhexine combined with SOC in PCR negative result (SARS-CoV-2 negative from baseline); To evaluate the clinical efficacy Bromhexine combined with SOC in reducing the symptoms of COVID-19 disease; To evaluate the efficacy of Bromhexine combined with SOC on the need for medical care, hospitalization, oxygen therapy, or mortality through day 28 from baseline; To evaluate the safety of Bromhexine administered in patients infected with SARS-CoV-2.

Methodology:

A phase 3, prospective, randomized, open-label, standard-of-care controlled, parallel-group, multicentre, drug repositioning clinical trial, with allocation ratio 1:1 of study groups Bromhexine plus SOC versus SOC group.

The random allocation sequence was generated by an independent technician, in random blocks of 4 and 6 treatments to distribute 10 randomization number envelopes by centre. The allocation concealment was done by electronic database monitoring. The investigators selected the study patients and assigned the study interventions opening the randomization envelopes after the signed informed consent was obtained. The statistician was blinded to the study group during the study analysis.

The patients must be diagnosed of active SARS-CoV-2 infection confirmed by compatible symptoms and a positive result in the detection tests for active infection (DTAI), rapid antigen detection test or in the PCR for viral RNA detection test.

The stratification of asymptomatic group of patients was discarded due to restrictions for the use of DTAI or PCR test in Spain.

The study setting was consultation in Primary Health Care of 13 centres located in Madrid (Spain).

Two randomised, open-label study group of patients were included to receive treatment with Bromhexine 3 daily doses of 10 ml

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(48 mg/day) for 7 days, plus SOC treatment versus the SOC treatment study group. All the patients were followed up to 28 days. Phone contacts were completed at day 1, 4, 7 and 14 and a visit at day 28. In all the contact with the patient the pulse oximetry, heart rate and temperature were determined by the patient with the study material supplied, and questions about the appearance of new symptoms and the symptoms severity was assessed in a ten-point severity scale.

The viral load was determined by detection of three highly conserved regions of specific genes of the SARS-CoV-2 pathogenic viral RNA strain (ORF1ab, N Protein and S Protein), in saliva samples obtained at baseline, day 4, day 7 and day 14 form study treatment initiation. All the study samples were analysed in a central laboratory (ARQUIMEA,Medical, S.L.U.). Viral RNA was obtained using the Chemagic Viral DNA/RNA 300 kit H96 (Perkin Elmer), purification of viral RNA was done using the automated Chemagic 360 Instrument (Perkin Elmer). RT-qPCR was completed with the TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermofisher) and detection of ORF1ab, N Protein and S Protein regions of the SARS-Cov-2 virus were completed in the 7500 Real-Time PCR instrument (Thermofisher) and QuantStudio 5 Real-Time PCR instrument (Thermofisher). The sensitivity of the platform was >99% and specificity of 99.5%.

The primary efficacy endpoint was to compare the reduction in viral load as Basal-4 days difference in SARS-CoV-2 RT-qPCR number of cycles of the Bromhexine in combination with SOC group versus the SOC group as an assessment of efficacy at 4 days from baseline. A higher number of cycles mean a lower viral load.

The secondary efficacy endpoints in the comparison of the group of patients treated with Bromhexine or with SOC were pre-defined as: Comparison of the reduction in viral load (Basal-7 days difference, basal-14 days difference, in SARS-CoV-2 number of Cycles) as an assessment of efficacy at 7 and 14 days from baseline; Comparison of the proportion of patients with negative SARS-CoV-2 PCR result at 4, 7, and 14 days from baseline. Positive values are considered if at least 2 of the 3 genes analysed amplify with cycles below the mean value of 35 (Ct<35); Comparison of the time from baseline to obtain a negative PCR test for SARS-CoV-2; Comparison of the clinical efficacy in reducing the symptoms of COVID-19 disease was assessed: Reduction in severity of each symptom (evaluated by 0 to 10 point numerical rating scale NRS) related to SARS-CoV-2 disease at 4, 7, 14, and 28 days or EOS from baseline; Proportion of patients with clinical improvement, defined as improvement by two or more points on a 0 to 10 NRS; Time to clinical improvement; Proportion of patients in whom each symptom related to SARS-CoV-2 disease disappears at 4, 7, 14, and 28 days from baseline; Proportion of patients with no symptoms at 4, 7, 14 and 28 days from baseline; Time to disappearance of each symptom related to SARS-CoV-2 disease from baseline; Comparison of the need for medical care, hospitalization, oxygen therapy, or mortality through day 28 from baseline: Proportion of patients requiring hospitalization, oxygen therapy, or who develop complications from SARS-CoV-2 disease through Day 28 from baseline; Proportion of patients who die within the 28-day follow-up period and reported mortality within three months of study end; To evaluate the safety of Bromhexine in patients with COVID-19.

Number of patients (planned and analysed):
200 planned; 193 enrolled; 191 analysed

Diagnosis and main criteria for inclusion:

The patients must be diagnosed of active SARS-CoV-2 infection confirmed by compatible symptoms (fever, cough, shortness of breath or difficulty breathing, sore throat, body or muscle pain, fatigue, headache, chills, nasal congestion, loss of taste or smell, nausea or vomiting, diarrhoea) and a positive result in the detection tests for active infection (DTAI), rapid antigen detection test or in the PCR for viral RNA detection test.

Inclusion criteria:1) Patient, of either sex, 18 years of age or older; 2) Patient with a diagnosis of active SARS-CoV-2 infection confirmed by Compatible symptoms and a positive result in the detection tests for active infection (DTAI), rapid antigen detection test or in the PCR for viral RNA detection test; 3) Patients presenting with symptoms must present within the last 72 hours with one or more of the following symptoms associated with SASR-Cov-2 infection: fever, cough, shortness of breath or breathing difficulties, sore throat, body or muscle pain, fatigue, headache; 4) In patients with symptoms due to SARS-CoV-2 infection, the severity of symptoms should be mild or moderate; 5) The patient has received sufficient information about the study orally and in writing and has signed the informed consent to participate in the clinical trial.

Exclusion criteria: 1) Patient living with a patient who has previously been enrolled in the study and who continues to be followed up (until Day 28); 2) Patient with severe COVID-19 disease; 3) Patient has any of the following diseases that may be affected or affect the results of the study: Active infectious disease other than SARS-CoV-2 infection requiring systemic therapy; Clinically significant uncontrolled respiratory disease; Prior cardiovascular disease: ischemic heart disease, heart failure, atrial fibrillation; Severe kidney failure; Active or treated malignancy; Immunosuppression due to an illness or the treatment received; Illness requiring surgical intervention within 30 days following the screening for the study; Severe obesity; Other diseases that the physician believes may pose an increased risk to the patient;4) Patient with hypersensitivity or intolerance to Bromhexine or to any of the excipients; 5) Pregnant women; 6) Breast-feeding women; 7) A patient with mental impairment that invalidates his/her

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ability to consent to participate in the study, or that limits his/her ability to comply with the study requirements, or that is expected to be non-cooperative; 8) Patient on treatment with drugs with known antiviral potential; 9) Participation in a clinical trial within the last month.

Test product, product dose and mode of administration, batch number:

Bromhexine 16 mg (10 mL) three times a day (48 mg/day) for 7 days, given before meals (breakfast, lunch, and dinner); Batch number: 200742; Expiry date: June 30th, 2023

Plus Standard of Care for SARS-CoV-2 disease.

Duration of treatment:

7 days.

Reference therapy, dose and mode of administration, batch number:

Standard of care treatment (SOC) for SARS-CoV-2 disease: acetaminophen 500 mg 1-4 times daily, non-steroidal anti-inflammatory drugs, symptomatic treatment, and hydration for mild clinical conditions. Only if suspected bacterial co-infection/superinfection should be prescribed azithromycin 500 mg/24 h oral for 3 days plus amoxicillin 1g/12 hours for 7 days, or amoxicillin/clavulanic acid 875 mg/125 mg every 8 hour for 7 days; alternatively, levofloxacin 500 mg every 12 hours on the first day and 500 mg every 24 hour for 4 days.

Statistical methods:

The null hypothesis considers no difference between the group of patients treated with Bromhexine combined with SOC and the group treated with SOC alone in the reduction in viral load (number of RNA replicates of SARS-CoV-2 virus determined by PCR in saliva samples) from the baseline visit prior to treatment and Day 4 post-treatment. The sample size calculation for the primary study variable was performed for a two-sided analysis of variance, with fixed effects and two levels in the factor evaluated corresponding to treatment or control group. A Type I error is set at a two-sided 0.05 level with a minimal effect with clinical relevance of 2 log10 reduction in viral copy number as the minimal difference between the on-treatment groups. A moderate effect of 0.25 (Cohen's f) was targeted leading to an expected common standard deviation of 4 log10. Given a sample of 200 patients (100 assigned to receive the evaluated treatment and 100 to receive SOC), a power of 94% is obtained to demonstrate the estimated difference. (Sample Power, IBM-SPSS).

The main analysis of the primary endpoint was measured by the Student t test for independent samples. The ANOVA for repeated measurements and a factor (Split-Plot) with Bonferroni adjustment for multiple comparisons was applied to the comparison of viral load between the study groups at baseline, day 4, day 7 and day 14. The primary analysis was adjusted based on justified demographic and effect-modifying variables. Kaplan Meier survival analysis and Log Rank test was applied for the analysis of time to get negative PCR. Type I error is set at a two-sided 0.05 level. The software IBM-SPSS 27.0 was used for the statistical analysis.

Summary - Conclusions

A final sample of 193 patients (191 evaluable) was included in 13 study centres with distribution of 98 patients in the Bromhexine + SOC treatment group and 93 patients in the SOC treatment group. A total of 127 patients (66.5%) were female and 64 males (33.5%), with mean age of 47.8 years old (SD 1.1), being 179 (93.7%) Caucasian and 12 Hispanic (6.3%). 37 patients (19.4%) suffered previous COVID-19 disease a mean of 16.3 months (SD 1.4) before the study initiation.

The number of patients with previous complete vaccination for SARS-CoV-2 was 182 (95.3%), with a mean of 5.3 months (SD 0.23) from last vaccination dose. The number of patients with previous COVID-19 infection was 19.4% (37 patients), 16.3 months (SD 1.37) from study baseline visit.

A total of 179 patients (95.9%) were included with mild COVID-19 disease, 7 patients (3.7%) had moderate disease and 5 patients (2.6%) were asymptomatic.

Baseline patient's demographic anthropometric and clinical history were homogeneous between the study groups. A mean of 6.1 symptoms (SD 0.29) were present at baseline observing 0 to 19 symptoms at baseline. No differences in the number or symptoms severity were observed between the study groups at baseline except for nasal congestion that was more severe in the SOC group (6.4 points) versus the Bromhexine plus SOC (5.3 points), p=0.008. Also, the otalgia was more severe for the SOC group (7 points) versus the intensity in the Bromhexine plus SOC group (3.8 points), 0.047. Vital signs, systolic and diastolic blood pressure and respiratory rate, heart rate, oxygen saturation and temperature were also homogeneous between the study groups. Viral load at baseline was homogeneous between the study groups for ORF1ab (22.5 Cts, SD 0.6), N Protein (22.8 Cts, SD 0.6) and S Protein (41.7 Cts, SD 2.4). 187 patients (97.9%) completed the 28 days of follow-up.

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Efficacy Results:

For the primary efficacy endpoint, as reductions in viral load (increasement of number of Cycles) from baseline to Day 4 of follow-up, no statistically significant differences were observed for none of the three specific genes of the SARS-CoV-2 pathogenic viral RNA strain with mean difference of 0.9 Cts (95%CI -6.7-8.5) for ORF1ab (p=0.817), mean difference of 1.3 Cts (95%CI -6.4-3.7) for N Protein (p=0.603) and mean difference of 4 Cts (95%CI -4.3-12.4) for S Protein (p=0.340).

For ORF1ab, significant increasement in Cts (reductions in viral load) were observed during the follow up for the total group from baseline to any time-point of the follow-up (p<0.001), but no differences were observed between the study groups at day 4 (p=0.765), Day 7 (p=0.431) and Day 14 (p=0.163). For N Protein, significant increasement in Cts were observed during the follow up for the total group from baseline to any time-point of the follow-up (p<0.001), but no differences were observed between the study groups at day 4 (p=0.678), Day 7 (p=0.961) and Day 14 (p=0.583). For S Protein, significant increasement in Cts were observed during the follow up for the total group from baseline to any time-point of the follow-up (p<0.001), but no differences were observed between the study groups at day 4 (p=0.592), Day 7 (p=0.450) and Day 14 (p=0.124).

A sensitivity analysis was done on the primary efficacy endpoint and in the evolution of the viral load with non-imputation rules applied to the study data, with no differences with the results of the main analysis.

No significant differences were observed between the Bromhexine + SOC versus SOC study group in the percentage of positive PCR results at Day 4, of 86.7% vs 80.6% (p=0.254), at Day 7 of 74.5% vs 65.6% (p=0.179) and Day 14 of 53.1% vs 61.3% (p=0.251), neither in the viral load classification (low, medium, high) in the follow-up.

Median to get the negative result was 14 days (12.2-15.8), with no statistical differences between the study groups (0.565).

No significant differences between the study groups were observed in the evolution of the vital signs that significantly improved form day 1 to day 28 (p<0.05) in the oxygen saturation (%), heart rate (p<001) and axillary temperature (p<0.001).

No statistical differences were observed between the study groups in the severity of any or the symptoms observed at Day 1, Day 7, Day 14 and Day 28, except for the dysgeusia at Day 4 with 1.6 points vs 3 points (p=0.005) and arthralgia with 1.7 points vs 2.4 points (p=0.014) more intense in the SOC study group.

A total of 38 patients (19.9%) continued with persistent symptoms after the day 28, with no differences between the study groups.

Patients with previous SARS-CoV-2 infection showed lower viral load at Day 0 and during the follow-up compared to patients with no previous COVID-19 disease. This difference was not observed on the vaccinated versus non vaccinated patients.

Safety Results:

A total of 13 patients (6.8%) experienced adverse events, 8 patients in the Bromhexine plus SOC group (8.2%) and 5 patients in the SOC group (5.4%), with no statistical differences (p=0.445). The total number of adverse events observed was 17, 64.7% (11) mild, 23.5% (4) moderate. Not related severe dizziness in one patient (5.9%) and one serious pulmonary thromboembolism (5.9%) were observed.

Three adverse events were considered related to Bromhexine (dizziness, nausea, and pasty mouth), 2 possibly related (constipation and tinnitus) and one unknown (pruritus), with 11 adverse events not related to the study treatment (64.7%). No adverse event led to premature discontinuation of the study drug. Two moderate treatment-emergent laboratory abnormalities were observed in the Bromhexine plus SOC study group, but were considered as not related (leucocyte elevation, transaminase elevation).

One patient of the SOC group needed to be hospitalized due to the COVID-19 disease, also needing oxygen-therapy, 12 days after the initiation of the study.

Conclusion

No significant differences were observed between the study groups in the viral load at Day 4 (ORF1ab, N Protein, S Protein), nor in the secondary efficacy endpoints. In this study cannot be demonstrated arguments in favour of the use of Bromhexine for the treatment of mild to moderate SARS-CoV-2 infection.

Date of report
September 15th, 2022