COVID-19: Azelastine nasal spray Reduces Virus-load In Nasal swabs (CARVIN). Early intervention with azelastine nasal sprays reduces viral load in SARS-CoV-2 infected patients. First report on a double-blind placebo-controlled phase II clinical trial.

Jens Peter Klussmann
University of Cologne, Center for Molecular Medicine Cologne (CMMC), Faculty of Medicine and University Hospital, Cologne, Germany

Clara Lehmann
University of Cologne, Center for Molecular Medicine Cologne (CMMC), Faculty of Medicine and University Hospital, Cologne, Germany

Maria Grosheva
University of Cologne, Medical Faculty, Department of Otorhinolaryngology, Head and Neck Surgery, Cologne, Germany

Kurtulus Sahin
Clinstat GmbH, Max-Planck-Str. 22, 50858 Cologne, Germany

Eszter Nagy
CEBINA GmbH, Karl-Farkas-Gasse 22, 1030 Vienna, Austria

Valéria Szijártó
CEBINA GmbH, Karl-Farkas-Gasse 22, 1030 Vienna, Austria

Gábor Nagy
CEBINA GmbH, Karl-Farkas-Gasse 22, 1030 Vienna, Austria

Robert Konrat
Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria

Peter Meiser
URSAPHARM Arzneimittel GmbH, Industriestraße 35, 66129 Saarbruecken, Germany

Michael Flegel
URSAPHARM Arzneimittel GmbH, Industriestraße 35, 66129 Saarbruecken, Germany

Paula Aguiar de Aragão
University of Cologne, Medical Faculty, Department of Otorhinolaryngology, Head and Neck Surgery, Cologne, Germany

Henning Morr
University of Cologne, Medical Faculty, Department of Otorhinolaryngology, Head and Neck Surgery, Cologne, Germany

Helal Al Saleh

University of Cologne, Medical Faculty, Department of Otorhinolaryngology, Head and Neck Surgery, Cologne, Germany

Belisa Russo

ClinCompetence Cologne GmbH, Theodor-Heuss-Ring 14, 50668 Cologne, Germany

Susanne Müller-Scholtz

ClinCompetence Cologne GmbH, Theodor-Heuss-Ring 14, 50668 Cologne, Germany

Silke Allekotte

ClinCompetence Cologne GmbH, Theodor-Heuss-Ring 14, 50668 Cologne, Germany

Ralph Moesges (ralph.moesges@uni-koeln.de)

University of Cologne, Institute of Medical Statistics and Computational Biology (IMSB), Faculty of Medicine, Kerpener Str. 62, 50937 Cologne

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Research Article

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Abstract

Background:

The current COVID-19 pandemic has had a major influence on our daily lives. The most frequent early symptoms associated with SARS-CoV-2 infection are coughing, fever, rhinitis, and loss of smell and taste. If the infection progresses to the lower respiratory tract, it can cause massive inflammation of the pulmonary system, which can be life threatening. There is urgent need for a broadly available and effective therapy for the treatment of early infections with SARS-CoV-2 in order to prevent progression to severe disease.

Methodology:

CARVIN is a phase II proof of concept, randomized, parallel, double-blind, placebo-controlled, interventional clinical trial. 90 SARS-CoV-2 positive volunteers were randomized into three groups to receive either placebo, azelastine 0.02% or azelastine 0.1% nasal spray for a period of 11 days. Seven nasopharyngeal swabs were taken during this period for quantitative PCR measurements assessing the viral load via the ORF 1a/b and E genes. Investigators also assessed patients' status continuously throughout the trial, and the intensity of individual symptoms were reported by the patients using an electronic diary. Two safety follow-ups were performed at days 16 and 60 of study participation.

Results:

Since the data of the primary outcome did not show a normal distribution, all statistical tests presented here were done non-parametrically and all p-values are descriptive and without adjustment for multiple testing. A broader descriptive analysis will be performed at a later date on all variables and it will be published in a peer-reviewed publication. A wide range of initial viral loads in the nasopharyngeal swabs of the study population was observed with an overall median/mean + SD Ct value of approximately 21.9 / 23.6 $\pm$ 5.8, corresponding to $\log_{10}$ 6.6 $\pm$ 1.8 copies per /ml. Out of the 90 enrolled subjects, at least 54 carried the Alpha (B.1.1.7, UK) variant.

Treatment with azelastine nasal sprays resulted in a greater but non-significant decrease in mean viral load compared to that measured in the placebo group at all 6 timepoints after initiation of treatment. This tendency was stable and most pronounced on day 8 (after 7 days treatment), when in the 0.1% and 0.02% azelastine nasal spray groups, an approximately 8- and 29-fold greater clinically meaningful reduction of the baseline viral load, respectively, compared to placebo was observed (based on the ORF1a/b gene). On days 4 and 11, approximately 4-fold greater mean viral load reduction was seen in the 0.1% azelastine group.

Differences in mean viral load compared to baseline values were seen starting on the second day (after one day of treatment) in the azelastine 0.1% and azelastine 0.02% group for ORF 1a/b gene, and with azelastine 0.1% for the E gene, while this reduction was less pronounced in the placebo group.

The effects of 0.1% azelastine nasal spray treatment to accelerate viral load reduction were even more pronounced in patients with initial high viral load (subgroup analyses in patients exhibiting initial Ct values below 25 and below 20, respectively). Of note, by day 8 the PCR-test had turned negative in more patients in the 0.1% azelastine group (n=6, p= 0.01 for the ORF 1a/b gene and n = 3, p= 0.08 for the E gene) and in the 0.02%

azelastine group (n=8, p< 0.01 for the ORF 1a/b gene and n = 5, p= 0.02 for the E gene) than in the placebo group (n=0 for the ORF 1a/b gene and n = 0, for the E gene).

Discussion:

This study provides the first clinical hints of the effects of an azelastine nasal spray in SARS-CoV-2 positive patients. Subgroup analyses performed in patients exhibiting high initial viral loads are further suggestive of azelastine's potential as an antiviral treatment.

Introduction

Corona viruses are single-stranded RNA viruses that belong to the family *Coronaviridae*, subfamily *Coronavirinae*. Only seven species are human-pathogenic: NL63 (HCoV-NL63), 229E (HCoV-229E OC43), (HCoV-OC43), HKU1 (HCoV-HKU1), severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV). While the first four species listed above cause common cold, SARS-CoV and MERS-CoV have caused outbreaks characterized by severe invasive infections with high mortality rates, starting in 2002 and 2012, respectively [1, 2]. The current COVID-19 pandemic is associated with SARS-CoV-2, which has emerged from the Wuhan region of China at the end of 2019. It is characterized by the emergence of novel variants with higher transmissibility and/or virulence. The most frequent symptoms regarding the COVID-19 pandemic are coughing, fever, rhinitis, and loss of taste and smell, which can persist for several months [3]. Less frequent symptoms are sore throat, shortness of breath, headaches and aching limbs, loss of appetite, weight loss, nausea, abdominal pain, vomiting, diarrhoea, conjunctivitis, skin rash, swelling of lymph nodes, apathy, and somnolence. In more severe cases, SARS-CoV-2 infection proceeds along the respiratory tract reaching the lungs, which can cause massive inflammation with respiratory distress and systemic consequences, which may be life threatening.

It is known that the main route of infection for SARS-CoV-2 is the respiratory uptake of virus-containing particles produced by breathing, coughing, speaking, singing and sneezing [4]. Since viral levels during early infection tend to be highest in the nose and nasopharynx, a nasal spray with an active substance that inhibits virus replication may be able to stop the progression of the disease to the lower respiratory system and also reduce the transmission of the virus to an uninfected individual.

Azelastine hydrochloride nasal spray is an approved medicinal product that has been marketed globally for decades. In general, it is used to relieve symptoms of allergic rhinitis (runny or stuffy nose) at a concentration of 0.1% w/v. The active substance (azelastine hydrochloride) is an histamine-1 receptor antagonist, which also shows anti-inflammatory effects, via mast cell stabilization and inhibition of leukotriene and pro-inflammatory cytokine production [5, 6]. Azelastine inhibits TNF-alpha release, granulocyte macrophage colony-stimulating factor generation and is able to reduce levels of a range of inflammatory cytokines, e.g., IL-1beta, IL-4, IL-6 and IL-8, cytokines that have a significant role in the inflammatory response [7].

Several independent *in vitro* laboratory and epidemiological studies have indicated azelastine's potential to efficiently reduce SARS-CoV-2 viral load and infection rates [8–13]. From a total of 1,800 approved drugs screened *in vitro* by use of a SARS-CoV-2-S pseudovirus entry inhibitor model, fifteen drugs were identified as active inhibitors, but only 7 of these drugs were identified as active against SARS-CoV-2, three of which were

anti-histamines: clemastine, trimeprazine and azelastine hydrochloride [8]. Another study published in 2021, showed that use of antihistamines such as loratadine, diphenhydramine, cetirizine, hydroxyzine, and azelastine was associated with reduced incidence of positive SARS-CoV-2 test results. This retrospective data base survey study was performed using a total of 219,000 medical records, and antiviral activity was verified in cell culture [9].

In a collaborative project, CEBINA (Central European Biotech Incubator and Accelerator/Vienna) demonstrated that azelastine had pronounced anti-SARS-CoV-2 activity *in vitro* in Vero E6 cell cultures. This was observed at an EC_{50} of ~ 6 µM which is an approximately 400-fold lower concentration compared to commercially available azelastine nasal sprays. In a highly relevant and translational *in vitro* model using reconstituted human nasal tissue, a five-fold diluted commercially available azelastine nasal spray solution inhibited viral replication by 99.9% within 72 hours after SARS-CoV-2 infection [13].

Furthermore, 3 independent groups predicted interaction of azelastine hydrochloride with the main protease of SARS-CoV-2: Mpro or 3CLpro [10–12]. Ghahremanpour et al. also provided experimental evidence for the inhibition of the enzyme in a kinetic activity assay [10].

Reznikov L.K. 2021 also showed that azelastine has anti-viral properties in cell culture, using azelastine as off-target for the ACE2 and the sigma-1 receptor [9].

The aim of our study was therefore to confirm the preclinical evidence for azelastine's antiviral activity in patients tested positive for SARS-CoV-2 in nasopharyngeal swabs. Reducing the infection of the nasal mucosa by local treatment with azelastine nasal spray may lower the viral load and consequently limit the progression of the infection to the lower respiratory tract as well as transmission of the pathogen.

Patients And Methods

Study Setting

This trial was conducted at the Department of Otorhinolaryngology, Head and Neck Surgery of the Faculty of Medicine of the University of Cologne, Germany. Outpatients visiting Corona test centres were informed about the possibility of participating in the current trial. Patients aged 18 to 60 years were eligible to participate if tested positive for SARS-CoV-2 within 48 hours prior to inclusion into the trial and had to quarantine at home due to instructions of the local health authority. A complete list of all inclusion and exclusion criteria is presented in Table 2. Patients were visited in their homes on regular basis by the investigators, physicians specialised in otorhinolaryngology, medical hygiene or general medicine.

Study Design

This is a prospective, randomized, double-blind, placebo-controlled dose-finding study, in which azelastine nasal spray was used in 2 doses: the commercially available concentration of 0.1% and a 5-fold lower concentration of 0.02%. After having given informed consent, patients tested positively for SARS-CoV-2 were examined to

assess eligibility according to inclusion/non-inclusion criteria and subsequently randomized to one of the three study groups.

The first administration of the nasal spray was carried out in the presence of the investigator, products were subsequently self-administered for the following period of 11 days (treatment phase). During the treatment phase, 7 visits (V1-V7) took place: on day 1, day 2, day 3, day 4, day 5, day 8 and day 11. Samples taken on day 1 represent pre-treatment samples. During these visits, nasopharyngeal swabs were taken to perform quantitative PCR measurements, and the investigators assessed the patient status in accordance with the WHO clinical progression scale [(14)]. Additionally, safety follow-ups were performed at two time-points. On day 16, an on-site visit (V8) for female patients was conducted in order to perform a urine pregnancy test and to assess the safety of the therapy. For male patients, the assessment was done via phone call. A final safety follow-up as well as assessment of the patient status (WHO scale) by a phone call was done on day 60 (V9) for all patients.

Patient reported outcomes were documented both by patient diaries and questionnaires. Therefore, during the treatment phase, the patients were required to document the severity of their COVID-19 related symptoms in an electronic diary on a daily basis. Moreover, on day 1, day 5, day 8 and day 11, patients completed the standardized SF-12 questionnaire of quality of life. A summary of activities performed during the study is displayed in Table 1.

Randomization

Assignment of the treatment with the investigational medicinal product (IMP) in the different doses vs. placebo to each treatment number was performed in a centrally conducted, computer-generated 1:1:1 randomization procedure. Treatment kits were manufactured by URSAPHARM Arzneimittel GmbH, Saarbruecken, Germany, according to the randomization list. Patients were assigned a treatment number in an ascending mode according to their chronological order of inclusion.

Blinding

CARVIN is a double-blind study. The list with the assignment of treatment number was kept at the URSAPHARM production facility until the end of the trial. No person involved in the conduct of the study will know the treatment of individual patients before unblinding following a Data Review Meeting. In addition to the treatment kits, sealed emergency envelops were delivered to the trial site. The treatment sequence of each treatment number was stored in emergency envelopes. A copy of these emergency envelopes was kept by the sponsor’s safety department. Treatment assignment code was only to be broken in exceptional cases.

Intervention and comparator

The trial medication was manufactured at URSAPHARM Arzneimittel GmbH. Participants were randomised to one of three groups: placebo nasal spray, azelastine 0.02% nasal spray or azelastine 0.1% nasal spray (the latter

being identical to the commercial product Pollival®). All nasal sprays were composed of hypromellose, disodium edetate, citric acid, disodium phosphate dodecahydrate, sodium chloride and purified water. Additionally, azelastine 0.02% nasal spray and azelastine 0.1% nasal spray contained 0.2 mg/ml or 1 mg/ml azelastine hydrochloride, respectively. Treatment was administered during a period of 11 days after inclusion in the study. Administration was done with one puff per nostril, 3 times a day.

Nasopharyngeal swabs

Nasopharyngeal swabs were obtained using nylon-flocked swabs (Biocomma; SW01E, flexible minitip). Following sampling, swabs were placed into 3 mL Virus Transport Medium (VTM, Biocomma) and delivered to the laboratory as quickly as possible. If delivery took place within 24h after sampling, samples were to be stored at <25°C, if storage period was greater than 24h (e.g., on Sundays), samples had to be stored and shipped at 2-8 °C. Samples were processed on the day of receipt at the central processing laboratory (Institute of Virology, University Hospital Cologne) by vortexing and aliquoting the viral transport medium, and stored at -80°C until analysis.

Quantitative PCR

SARS-CoV-2 RNA levels in nasopharyngeal swabs were determined by quantitative RT-PCR using the cobas® SARS-CoV-2 Test on the cobas® 6800 system (Roche Diagnostic, Mannheim, Germany). For quantification of SARS-CoV-2-RNA in copies/ml, a standard curve derived from a dilution series of a SARS-CoV-2 cell culture isolate in VTM and adjusted to Ct values obtained from two samples with defined SARS-CoV-2-RNA copy numbers (10^6 and 10^5 copies/ml; INSTAND e.V., Düsseldorf Germany) was used. For calibration purposes of quantitative assessments, reference samples were included with each PCR run. The dual-target RT-PCR independently targets the ORF1a/b and the sarbecovirus E genes, and assays were considered positive if at least one target returned a positive result. Information on individual variants was obtained through the original laboratory reports, when available. Detection of the B.1.1.7 variant was based on positivity of the mutation N501Y and H69/80V deletion.

Patient reported outcomes

Patients were required to document their COVID-19 specific symptoms on a daily basis in an electronic patient diary. The following parameters were evaluated on a 5-point scale from 1=symptom present very weakly to 5= symptom present very strongly: anosmia, ageusia, cough, sore throat, shortness of breath, coryza, general weakness, headache, aching limb, loss of appetite, pneumonia, nausea, abdominal pain, vomiting, diarrhea, conjunctivitis, rash, lymph node swelling, apathy, somnolence. In addition, presence or absence of fever (≥ 38.0 °C) was documented daily (0= no fever, 3= fever). Symptoms were analyzed separately as the means of single symptom scores, and the means of the total symptom score (TSS) reflecting the sum of all 20 single symptoms and presence/absence of fever. Sum scores were analyzed for this preliminary analysis, which could reach a

minimum value of 20 and maximum value of 103. The application of the study medication also had to be daily documented in the electronic diary.

In addition, patient's quality of life was evaluated by use of the SF-12 questionnaire, a shortened form of the SF-36 questionnaire, covering 12 items divided into the eight QoL domains ‘physical functioning’; ‘role limitations due to physical health’, ‘role limitations due to emotional problems’, ‘energy/fatigue’, ‘emotional well-being’, ‘social functioning’, ‘pain’, and ‘general health’ [14-16].

At the end of the study, patients and investigators assessed the overall tolerability and efficacy of the treatment as ‘very good’, ‘good’, ‘moderate’ or ‘poor’.

Patient status determination (these data are still blinded and are therefore not presented in this preliminary report)

The patient status was assessed by the investigators with a 11-category ordinal score proposed by the WHO [14, 15]. In addition, investigators measured body temperature (forehead) during V1-V7 and oxygen saturation of the blood (using a finger pulse oximeter) on V1, V3, and V5, V6 and V7.

Outcomes

The primary endpoint of the CARVIN study was to assess the clinical impact of the treatment with azelastine nasal spray in patients who had been positively tested for SARS-CoV-2. This was done by evaluating the virus load kinetics in individual patients and the median and mean virus loads of SARS-CoV-2 in the different treatment groups through quantitative PCR measurements from nasopharyngeal swabs.

The mean virus load (expressed in $\log_{10}$ cp/ml) during the treatment phase in the three study groups was obtained with PCR detection of both the ORF 1a/b and E genes and calculated from the respective Ct values using independent standard curves. Values were analysed separately for the entire data set as well as for subsets defined by Ct (Cycle threshold; indicating the number of PCR cycles necessary to detect a positive PCR signal) thresholds of 20 (Ct <20) and 25 (Ct<25).

Secondary endpoints to assess the clinical impact of the azelastine nasal spray included:

- Proportion of patients with decreased SARS-CoV-2 virus load
- Change in symptom severity (anosmia, ageusia, fever, cough, sore throat, shortness of breath, coryza, general weakness, aching limbs, loss of appetite, pneumonia, nausea, abdominal pain, vomiting, diarrhoea, conjunctivitis, rash, lymph node swelling, apathy, somnolence) from baseline.
- Change in patient state using a 11-category ordinal score as proposed by the WHO [17]
- Change in patient status by measurement of temperature and oxygen saturation of the blood
- Change in the quality of life reported in the SF-12 (shortened form of the SF-36) generic quality of life questionnaires

- Safety assessment (adverse events, including worsening of patient status/ symptoms)

The outcomes of the quantitative PCR-tests and the symptom scores documented in the electronic diaries presented in this first report were calculated based on the study protocol. A broader descriptive analysis will be performed after blinding is broken on all parameters and will be published in a peer-reviewed journal.

Statistical hypotheses

For this study, the data was analysed exploratively. There is no formal testing of a given hypothesis.

Sample size determination

The sample size calculation was based on the expected reduction of virus load during the treatment.

It was assumed that all treatment groups present identical baseline virus load at enrolment with a mean value of 5.5 $\log_{10}$ copies /mL ± 3 SD [18, 19]. Since azelastine has been shown to inhibit viral replication by 99.9% in Vero E6 cell culture and in reconstituted human nasal tissue cultures, it was assumed that a reduction of 3-log in virus load would be seen within 3 days in actively treated patients, while no effect on virus load reduction would be seen in placebo treated patients. Assuming a pooled standard deviation of $\sigma = 3$ units, a two-sided $\alpha = 0.05$ and a power of 90%, a sample size of 23 patients per treatment group was calculated. Anticipating a drop-out rate of 20%, the aim was to randomize 90 patients in total (30 patients per treatment group) to result in 23 patients per treatment group completing the study and being eligible for analysis.

Descriptive statistics

Both descriptive and exploratory statistics were performed. Subgroups were analysed exploratorily (e.g., subgroups regarding gender, age, symptom severity, etc.).

Continuous data were described by statistical estimates (mean, standard deviation, median, Q1, Q3, minimum, and maximum values).

Categorical data were described by absolute frequencies and percentage of valid cases.

The study endpoints were presented by descriptive statistics, and their changes from baseline (day 1) to day 11 were presented. Missing values were not replaced and no imputation rules were applied.

Significance tests

All tests were performed two-sided and the type 1 error (α) was set at 5%. The analysis aimed to compare the course of viral load between the three treatment groups. While comparison of categorial variables between groups were performed by Chi square testing, continuous variables were compared using ANCOVA with the factors baseline, visit, treatment group and treatment group visit. Changes within groups were analysed using a Wilcoxon signed rank test (against 0).

Preliminary analyses

A preliminary analysis of the efficacy and safety data was performed after the end of treatment for all included patients (day 11; follow-up of patients was continued until day 60). This included evaluation of patient's viral load and total symptom scores as well as adverse events observed until data lock for the preliminary analysis. All p-values presented hereafter are descriptive and not adjusted for multiple testing. Evaluation of individual signs and symptoms, oxygen saturation of the blood, SF-12, the WHO score including the patients' outcome on day 60 as well as a final safety and efficacy assessment (as described above) will be performed after the last visit of the last patient, and the comprehensive data will be published in a peer-reviewed journal.

The statistical analysis comprising all data available to that date was carried out by an external statistician who had access to the randomization list, while working separately from the study group, which remained blinded. The final analysis will also include the data collected during the safety follow-ups.

Results Of The Primary Analysis

Trial Population

In total 90 patients were recruited between March 9th and April 28th 2021. The preliminary results were calculated based on the Modified Intention-To-Treat set, including 28 patients in each of the treatment groups, after exclusion of 6 patients (2 per group) with all 7 PCR tests performed during the study being negative. These patients were considered to have an initial false positive PCR test externally performed before recruitment and inclusion into this trial.

The group of patients with baseline Ct values below 25 consisted of 19 patients in the 0.1% azelastine group, 21 patients in the 0.02% azelastine group and of 17 patients in the placebo group. The group of patients with baseline Ct values below 20 comprised 10 patients in the 0.1% azelastine group, 9 patients in the 0.02% azelastine group and 6 patients in the placebo group.

Primary outcome

The mean Ct value of the 84 evaluable subjects at enrollment was 23.6 +/- 5.8, corresponding to $\log_{10}$ 6.6 +/- 1.8 cp/ml (approximately 4 million viral copies per ml, the highest values being ~500 million cp/ml). Data on virus variants was available for 59 patients and 54 (92%) of those carried the alpha (B.1.1.7, UK) variant.

In general, there was a continuous decrease in the mean virus load in all 3 study groups, during the 11 days of treatment. When assessed by the ORF 1a/b gene, a reduction of virus load of $\log_{10}$ 4.16 was seen at the last day of treatment (day 11) compared to baseline (day 1) for the azelastine 0.1% group, of $\log_{10}$ 4.12 in the azelastine 0.02% and of $\log_{10}$ 3.55 in the placebo group (Figure 1). For the E gene, a reduction of $\log_{10}$ 5.46 was observed in the azelastine 0.1% group, of $\log_{10}$ 5.61 in the azelastine 0.02% and of $\log_{10}$ 5.08 in the placebo group (see Table 3). This reduction in the virus load was clinically meaningful for all three groups ($p<0.0001$) for both genes (see supplementary Table 3). Data of the primary outcome did not show a normal distribution (Shapiro-Wilk test, $p<0.05$). Therefore, the primary analysis for the viral loads was conducted non-parametrically and it did not show statistically significant differences between the treatment groups. This is in line with an ANCOVA analysis conducted thereafter which demonstrated significant values for the factors baseline and visit but not for treatment groups nor for the interaction of treatment groups with visits. However, when analysing individual values in more detail, for the ORF 1a/b gene a clinically relevant decrease in viral load was seen on the second day of the study (after 1 day treatment) in the azelastine 0.1% group ($p=0.0041$) and azelastine 0.02% group ($p=0.0428$). No such reduction was seen on this first day of the application of the spray in the placebo group *($p=0.2826$)*. For the E gene, a clinically relevant improvement was seen on the second day (after one day of treatment) in the azelastine 0.1% group ($p=0.0045$) and only one day later on the third day in the azelastine 0.02% group and placebo group (see Table S3).

By comparing the kinetics of mean viral load changes in the three groups (absolute changes from baseline), we observed greater reductions in the two azelastine containing nasal spray groups compared to placebo at the different test dates (see Figure 1), with the most pronounced differences on day 8 (after 7 days of treatment). Thus, on day 8, a viral load reduction (compared to baseline) of -3.08±2.58 ($\log_{10}$ cp/ml) in the 0.1% azelastine group, of -3.62±2.20 ($\log_{10}$ cp/ml) in the 0.02% azelastine group, and of -2.16±1.85 ($\log_{10}$ cp/ml) in the placebo group was observed. On days 4 and 11, an approximate 4-fold greater mean viral load reduction was seen in the 0.1% azelastine group compared to the placebo group. Of note, by day 8 the PCR-test had turned negative in more patients in the 0.1% azelastine group (n=6, $p=0.01$ for the ORF 1a/b gene and n=3, $p=0.08$ for the E gene) and in the 0.02% azelastine group (n=8, $p<0.01$ for the ORF 1a/b gene and n=5, $p=0.02$ for the E gene) than in the placebo group (n=0 for the ORF 1a/b gene and n=0 for the E gene). Absolute changes from baseline of PCR test positivity by visit are given in Table 5.

When analysing sub-groups of patients with an initial (baseline, before treatment) high viral load characterized by Ct values of less than 25 or less than 20 for both genes, we also observed greater reductions in viral load throughout the treatment period in the 0.1% azelastine nasal spray group compared to the placebo group (Figure 2, Table S1 & S2).

The analysis of the data subset with Ct values below 20 showed, by day 11, a reduction of the virus load of $\log_{10}$ 5.51 ($p=0.0020$) in the azelastine 0.1% group, $\log_{10}$ 4.03 ($p=0.0039$) in the azelastine 0.02% group and $\log_{10}$ 3.94 ($p=0.0313$) in the placebo group, based on the ORF 1a/b gene (see Table S1 & S3). With respect to the copy number of the E gene, a reduction of $\log_{10}$ 6.90 ($p=0.0020$) was seen in the azelastine 0.1% group, $\log_{10}$ 5.87 ($p=0.0039$) in the azelastine 0.02% group and $\log_{10}$ 6.20 ($p=0.0313$) in the placebo group from day 1 to day 11 (see Table S1 & S3). A clinically relevant effect of azelastine 0.1% on viral load reduction (log10 cp/ml) was seen by the second day for ORF 1a/b gene ($p=0.0059$) and E gene (0.0039). By the third day such a relevant reduction was also observed in the azelastine 0.02% group for the ORF 1a/b gene ($p=0.0078$) and E gene

(p=0.0195). A similar effect in the placebo group was not observed before the 4th day of treatment both for ORF 1a/b gene and for the E gene (p=0.0313, see Table S3). Detailed results of the data subset with Ct values below 25 are shown in the supplementary Table S2).

The comparison between treatment groups of the data subset Ct < 20 showed a more pronounced effect of the azelastine 0.1% group over placebo on day 8 regarding Ct and log10 cp/ml values of the ORF 1a/b gene (p=0.0197). Within the data subset Ct < 25, a similar difference between the azelastine 0.1% group and placebo was reached on day 4 regarding log10 cp/ml values of the ORF 1a/b gene (p=0.0495) and log10 cp/ml values of the E gene (p=0.0442). These data suggest that azelastine may accelerate viral clearance from the nasopharynx.

Main secondary outcome

One of the main secondary outcomes, the total symptom scores (Table 4) and changes during the course of the treatment phase (Figure 3) showed a reduction from baseline to day 11 in all study groups, the azelastine 0.1% group displaying the greatest improvement with 12.64 mean score reduction. The reduction of the symptom score from baseline to day 11 was -8.38 in the azelastine 0.02% group and of -10.50 in the placebo group. The reduction in the symptom score was clinically relevant for all three groups: p<0.001 for the azelastine 0.1% group and placebo group and p=0.0002 for the azelastine 0.02% group (see Table S4). A subgroup analysis for the patients with an initial CT value of less than 20 showed a reduction of -13.30 in the azelastine 0.1% group, -6.88 in the azelastine 0.02% group and of -11.67 in the placebo group. In this descriptive subgroup analysis, the reduction of the symptom score was more pronounced only for the azelastine 0.1% group on several days (see Table S4), however the size of these groups, in particular of the placebo was rather small (n=6). More detailed analyses are needed based on individual symptom scores to further assess the effect of azelastine treatment.

Safety

75 adverse events were reported by 72.4% of the patients in the azelastine 0.1% group, 79 adverse events were reported by 74.2% of the patients in the azelastine 0.02% group and 63 events were reported by 63.3% of the patients in the placebo group. No severe adverse events were reported during the treatment phase of this study. Detailed analysis of the observed adverse events will be presented in a subsequent publication, after the complete data review meeting has been performed.

Discussion

The preliminary results of this double-blind, placebo-controlled, randomized trial reported here suggest that azelastine hydrochloride 0.1% may be efficient in reducing the nasal viral load of non-hospitalized patients tested positively for SARS-CoV-2.

Our study population is characterized by an initial mean viral load of $\log_{10}$ 6.6 (~ 4 million) virus particle/ml (mean Ct value of ~ 24). This is one log higher than assumed at the study design in the autumn of 2020, based

on available literature. The potential reason for this, is the dominance of the alpha (UK, B.1.1.7) variant, during the enrollment phase (Spring 2021, Germany), that is known to infect the human nasal mucosa more efficiently than the wild type and has been associated with higher viral load [20]. Indeed, the majority of the study subjects carried this variant. Importantly, azelastine has been tested against the wild-type (D614G), alpha (B.1.1.7), beta (B1.1.351) and delta (B.1.617.2) variants of SARS-CoV-2 in Vero cells overexpressing the human ACE2 and transmembrane serine protease 4 (TMPRSS4) proteins and was found to have comparable potency against all these four variants (Konrat et al, unpublished data). Therefore, it is expected that data derived from our study - having patients infected mainly by the alpha, but also by the wild-type variants - can be used to extrapolate for the other major variants of concern tested *in vitro*, especially the delta variant that is the currently dominating and fast spreading variant worldwide.

We observed a gradual reduction of mean viral titers in the serially collected nasal swab specimen in all three study groups from baseline (day 1) to day 11 of treatment. Based on literature reports available by now, it must be expected that viral load reduction would be observed already as the consequence of natural viral clearance from the nasal mucosa during the study period, as most studies report an average approximately 2 weeks carriage of SARS-CoV-2. This stands in contrast to our initial assumptions and expectations when this study was planned. On the other hand, prolonged nasal positivity is reported, especially in symptomatic cases, and many other factors will have an influence on the individual viral load and clearance [21, 22]. We cannot rule out the possibility that the placebo (nasal spray buffer), applied three times a day also contributed to viral clearance. In a study conducted in the early 2000's, examining the potential effect of azelastine nasal spray usage on upper respiratory infections in children, it was found that the placebo group, receiving hypertonic saline solution (twice daily) also produced a favorable response compared to those receiving no treatment [23]. Recently, Shmuel *et al.* reported that a low pH hypromellose nasal powder spray containing common components of nasal sprays could reduce SARS-CoV-2 infection rate in an observational prospective open label user survey [24]. Importantly, we observed increased reduction of SARS-CoV-2 based on consistently lower viral copy numbers in nasopharyngeal swabs of subjects receiving azelastine 0.1% nasal spray during the 11-day treatment period, compared to the placebo group. The difference, however did not reach statistical significance in our population of 28 patients per group. Although one should be aware of this relatively small numbers of participants, particularly within the Ct < 20 group, the close patient supervision by physicians and the large quantity of performed PCR tests represent a strength of the current study.

It is important to note, that the results showed here, are representative of a first analysis done based on the study protocol, which can explain the small statistically significant differences. A broader analysis on all parameters documented in this trial is planned and will be performed, in which the intergroup differences will be explored.

Overall, our results are encouraging, particularly if considering recent SARS-CoV-2 vaccination and therapy study results [25]. Levine-Tiefenbrun *et al.* (2021) have shown that viral load was reduced in SARS-CoV-2 infections occurring 12–37 days after the first vaccination with BNT162b2 messenger RNA vaccine by 2.8 to 4.5-fold [26]. A recently published model that projects the effects of variants on disease transmission concluded that a 2 to 4-fold increase in nasal virus load increases transmission by up to 17% [27]. It has been known that the viral load is elevated by approximately 10-fold on average in the case of the B1.1.7 variant [28], which significantly increases the probability of transmission by up to 50% [29]. Therefore, reducing the viral load by 2 to 4-fold is likely to be meaningful to significantly reduce virus spreading.

A clinical trial investigating antibody therapies in non-hospitalized patients tested positive for SARS-CoV-2 infection demonstrated that treatment significantly decreased SARS-CoV-2 log viral load at day 11. Compared with placebo, the difference in the change in log viral load at day 11 was − 0.57 (p = 0.01) for a combination treatment with bamlanivimab and etesevimab [30]. Comparably, differences in reduction of viral load in our study with azelastine nasal spray were − 0.61 for the ORF 1a/b gene and − 0.38 for the E gene comparing treatment with azelastine 0.1% to placebo. Importantly, newly emerging variants have the potential to evade the immune response induced by natural infection or vaccination resulting in lower efficacy of protection from infection. The efficacy is especially affected in case of monoclonal antibody therapies, as it has been recently demonstrated with delta variant shown to be resistant against bamlanivimab, an approved COVID-19 therapeutic [31].

The current results also indicate that treatment with azelastine at the higher concentration (0.1%) might lead to an improvement in patients' symptoms. In our trial, this was observed particularly in patients with high viral burden (reflected by Ct values < 20), where clinically relevant improvement of total symptom scores (from baseline) was documented on day 6 and from day 8 on. The symptom score reduction observed with azelastine 0.1% treatment in this subset from 45.00 to 31.70 (day 1 to day 11) (p = 0.0195) was assessed as clinically meaningful. In the other groups (azelastine 0.02% and placebo) a reduction in the symptom scores were also observed, nonetheless, this decrease did not elicit a similar improvement. Obviously, these results must be considered preliminary due to the limited number of patients included, in particular for subgroup analyses. Assessment of single symptoms (which will be performed after the data review meeting) will be important and necessary to fully evaluate the potential benefit of the treatment with azelastine nasal spray.

Moreover, during the treatment phase with azelastine in both concentrations and placebo, no severe adverse events were reported. Therefore, treatment with azelastine appears safe in SARS-CoV-2 positive patients, which can be supported by the use of this substance in allergic patients for many decades. An additional safety analysis including the two safety follow-ups on day 16 and 60 will be performed soon, thereby completing the current preliminary study results.

Conclusion

Our preliminary results described here provide the first hint that azelastine hydrochloride nasal spray used in a 0.1% concentration may be effective in accelerating the reduction in the virus load in the nasal cavity and improving the general symptoms reported by COVID-19 patients. The positive effects on symptom improvement particularly in patients with high viral burden may indicate that azelastine hydrochloride nasal spray could be advantageous for this patient population. Additional analyses of the complete data set as well as future clinical studies will help understanding the impact of azelastine hydrochloride in treating SARS-CoV-2 infected patients.

Trial Status

CARVIN was enrolling patients under protocol version 1.1 dated 29th January 2021. Date of first enrolment: 09-03-2021, date of last patient last visit: 26-06-2021. Recruitment, treatment as well as safety follow-up phase is completed.

Declarations

ETHICS AND COMPETENT AUTHORITY APPROVAL AND CONSENT TO PARTICIPATION

Ethics approval was granted by the Ethics Committee of the Faculty of Medicine of Cologne University on the 10th of February 2021. Approval of the study by the German Federal Institute for Drugs and Medical Devices (BfArM) was given on 3rd February 2021.

Informed consent was obtained from all participants prior to involvement in the study.

STUDY REGISTRATION

The study was registered in the German Clinical Trial Register prior to inclusion of the first patient (DRKS00024520).

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AUTHORSHIP CONTRIBUTION

Eszter Nagy, Valéria Szijártó, Gábor Nagy and Michael Flegel contributed to the study design. Ralph Mösges, Susanne Müller-Scholtz, Silke Allekotte and Peter Meiser designed the study protocol. Belisa Russo drafted the manuscript. Jens Peter Klussmann was the principal investigator responsible for the conduct of the study. Paula Aguiar de Aragao, Henning Morr and Helal Al Saleh are investigators involved in the conduct of the study. All authors contributed to the preparation of the manuscript, read and approved the manuscript.

CONFLICT OF INTEREST

PM and MF are employed at URSAPHARM Arzneimittel GmbH. RM, SMS, BR and SA are employees of ClinCompetence Cologne, the CRO which organized this trial. EN, VS and GN are shareholders in CEBINA GmbH, RK and EN are inventors on related patent applications.

JPK and CL have received grants from the sponsor URSAPHARM Arzneimittel GmbH for performing this trial, KS, MG, PA, HM and HAS declare no conflict of interest.

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CORRESPONDING AUTHOR

Correspondence to Ralph Mösges.

Institute of Medical Statistics and Computational Biology (IMSB), Faculty of Medicine, University of Cologne, Kerpener Str. 62, 50937 Cologne, Germany.

Tel.: +49 221 7161 3323

Email: ralph@moesges.de

Tables

Table 1. Study Flow Chart

	Treatment Phase												Follow-up
Schedule (day)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**16**	**60**
Study visits	**V1**	**V2**	**V3**	**V4**	**V5**			**V6**			**V7**	**V8**	**V9**
Contact Study hotline	X												
Informed consent	X												
Inclusion & non-inclusion criteria	X												
Demographic data	X												
Temperature measurement	X	X	X	X	X			X			X		
Urine pregnancy test	X											X	
Oxygen saturation of the blood	X		X		X			X			X		
Sampling naso-pharyngeal swabs	X	X	X	X	X			X			X		
quantitative PCR measurement	X	X	X	X	X			X			X		
Assessment of Patient Status	X	X	X	X	X			X			X		X
Safety assessment (Patient’s AE profile)	X	X	X	X	X			X			X	X	X
Documentation of symptoms (patient)	X---------- ----------X												
Study drug administration	X---------- ----------X												
SF-12 QoL	X				X			X			X		
Final assessment											X		

Table 2. Inclusion and exclusion criteria for study participation

Inclusion criteria	Exclusion criteria
Legally competent patients capable of given informed consent	Hospitalization
Aged 18-60 years old	Simultaneous participation in other clinical trial or previous participation within 30 days before inclusion
Positive PCR test for SARS-CoV-2 (nasal swab taken no longer than 48h)	SARS-CoV-2 test older than 48h
Females: non-pregnant, non-lactating, with adequate contraception or unable to bear children	Relationship or dependence with the Sponsor, CRO and/or Investigator
	Risk of serious course of the disease (e.g. insulin-dependent diabetic patients, use of antihypertensive drugs)
	Inability to understand instructions/study documents
	Inability to administer the nasal spray
	Vulnerable patients: detained or committed to institutions by law court or legal authorities
	Females: pregnant, lactating, or of child-bearing potential and not using an adequate contraceptive method
	Concurrent anti-histamine therapy
	Concurrent nasal spray
	Contraindication for the use of azelastine (incl. hypersensitivity to the active substance or other ingredients)

Table 3. Quantitative PCR results (log10 cp/ml) for ORF 1a/b and E gene, during the course of the treatment phase (Modified Intention-To-Treat analysis set)

		Azelastine 0.1% (A)		Azelastine 0.02% (B)		Placebo (C)		Total	
		ORF1a/b	E	ORF1a/b	E	ORF1a/b	E	ORF1a/b	E
Day 1	N	28	28	28	28	28	28	84	84
	Missing	0	0	0	0	0	0	0	0
	Mean	6.52	6.60	7	7.08	6.30	6.41	6.61	6.70
	Std	1.94	2.02	1.27	1.34	2.11	2.16	1.81	1.88
	Median	6.91	6.97	7.35	7.45	7.09	7.18	7.09	7.17
	Min/Max	0/8.5	0/8.8	4.3/8.7	4.0/8.9	0/8.4	0/8.5	0/8.7	0/8.9
	Q1/Q3	5.4/8.1	5.5/8.3	6.1/8	6.2/8.2	5.4/7.7	5.6/7.8	5.6/7.9	5.7/8.1
Day 2	N	28	28	28	28	28	28	84	84
	Missing	0	0	0	0	0	0	0	0
	Mean	6.11	6.16	6.64	6.59	6.31	6.35	6.35	6.36
	Std	1.78	1.85	1.47	1.96	1.75	1.82	1.67	1.87
	Median	6.39	6.43	7.08	7.27	6.69	6.73	6.83	6.96
	Min/Max	0/8.7	0/8.8	3.9/9.0	0/0.91	0/8.4	0/8.7	0/9.0	0/9.1
	Q1/Q3	4.9/7.5	4.9/7.6	5.6/7.7	5.6/7.8	5.5/7.5	5.7/7.6	5.2/7.6	5.3/7.7
Day 3	N	28	28	28	28	28	28	84	84
	Missing	0	0	0	0	0	0	0	0
	Mean	5.93	5.87	6.32	6.39	5.87	5.88	6.04	6.05
	Std	1.70	2.06	1.81	1.88	1.77	1.89	1.75	1.94
	Median	6.33	6.44	6.85	7.02	6.15	6.25	6.38	6.49
	Min/Max	0/8.1	0/8.3	0/8.7	0/8.9	0/8.2	0/8.4	0/8.7	0/8.9
	Q1/Q3	4.9/7.1	4.9/7.3	5.4/7.6	5.3/7.7	4.7/7.1	4.6/7.2	4.8/7.3	4.8/7.4
Day 4	N	28	28	28	28	28	28	84	84
	Missing	0	0	0	0	0	0	0	0
	Mean	5.18	4.91	6	5.82	5.54	5.13	5.57	5.29
	Std	1.95	2.42	1.90	2.47	1.73	2.49	1.87	2.46
	Median	5.28	5.21	6.38	6.45	5.67	5.72	5.60	5.70
	Min/Max	0/7.9	0/8.2	0/8.9	0/9.1	0/7.9	0/8.0	0/8.9	0/9.1
	Q1/Q3	4.4/6.5	4/4.6	4.8/7.3	4.8/7.5	4.3/6.9	4.2/7	4.6/7	4.5/7.1
Day 5	N	28	28	28	28	28	28	84	84
	Missing	0	0	0	0	0	0	0	0
	Mean	4.82	4.78	5.21	4.75	5.04	4.75	5.02	4.76
	Std	1.94	2.01	1.97	2.61	1.87	2.36	1.91	2.31
	Median	5.19	5.26	5.40	5.34	5.42	5.51	5.39	5.38
	Min/Max	0/7.3	0/7.4	0/8.2	0/8.3	0/8.1	0/8.3	0/8.2	0/8.3
	Q1/Q3	4.1/6	3.7/6	4.1/6.8	3.7/6.9	3.9/6	3.6/6	4/6.3	3.6/6.4
Day 8	N	28	28	28	28	28	28	84	84
	Missing	0	0	0	0	0	0	0	0
	Mean	3.44	3.08	3.38	2.66	4.14	2.98	3.65	2.91
	Std	1.90	2.07	2.32	2.64	1.06	2.21	1.85	2.30
	Median	3.97	3.82	4.14	3.40	4.04	3.62	4.03	3.68
	Min/Max	0/6.0	0/6.1	0/6.4	0/6.6	0/5.8	0/6.0	0/6.4	0/6.6
	Q1/Q3	3.7/4.6	0/4.7	0/5.3	0/5.4	3.7/4.8	0/4.7	3.6/4.8	0/4.8
Day 11	N	28	28	28	28	28	28	84	84
	Missing	0	0	0	0	0	0	0	0
	Mean	2.36	1.15	2.87	1.47	2.75	1.33	2.66	1.31
	Std	2.12	1.86	2.12	2.22	1.80	1.82	2	1.95
	Median	3.60	0	2.66	0	3.73	0	3.68	0
	Min/Max	0/4.9	0/4.7	0/6.1	0/6.2	0/4.7	0/4.4	0/6.1	0/6.2
	Q1/Q3	0/4.1	0/3.7	0/4.4	0/4	0/3.9	0/3.5	0/4.1	0/3.7

Table 4. Summary of total symptom score by visit during the treatment phase (modified Intention-To-Treat analysis set)

		Azelastine 0.1% (A)	Azelastine 0.02% (B)	Placebo (C)	Total
Day 1	N	28	26	28	82
	Missing	0	2	0	2
	Mean	40.79	37.46	36.32	38.21
	Std	9.86	8.93	11.06	10.07
	Median	39.50	34.50	34.50	36
	Min/Max	23/61	23/57	21/60	21/60
	Q1/Q3	33.5/47.5	30/43	27/44.5	27/44.5
Day 2	N	28	28	28	84
	Missing	0	0	0	0
	Mean	38.89	37.29	33.96	36.71
	Std	10.57	9.22	12.13	10.78
	Median	37.50	36.50	32.50	36
	Min/Max	23/63	11/60	11/59	11/63
	Q1/Q3	31/45.5	32.5/42.5	25/40	29/43
Day 3	N	28	28	28	84
	Missing	0	0	0	0
	Mean	39.07	35.64	33.93	36.21
	Std	11.33	9.93	11.82	11.13
	Median	39.50	34	29	34
	Min/Max	23/64	15/62	21/61	15/64
	Q1/Q3	30/46	28/42	24.5/41	27.5/42.5
Day 4	N	28	28	28	84
	Missing	0	0	0	0
	Mean	38.11	35.64	33.39	35.50
	Std	10.38	9.22	11.70	10.54
	Median	38.50	33	31	33.50
	Min/Max	22/62	21/62	21/59	21/62
	Q1/Q3	30/42	28.5/40.5	24.5/36.5	27.5/41.5
Day 5	N	28	28	28	84
	Missing	0	0	0	0
	Mean	36.75	33.50	32.07	34.11
	Std	10.24	8.66	11.20	10.16
	Median	35	31.50	30	31.50
	Min/Max	21/59	21/60	21/61	21/61
	Q1/Q3	30/43.5	26/39	23/37.5	26/39.5
Day 6	N	27	28	28	83
	Missing	1	0	0	1
	Mean	35.19	31.75	31.21	32.69
	Std	10.98	9.43	11.08	10.54
	Median	32	31	28.50	31
	Min/Max	21/62	11/59	21/61	11/62
	Q1/Q3	26/42	25.5/37	22/34	25/37
Day 7	N	28	28	27	83
	Missing	0	0	1	1
	Mean	33.96	31.96	29.52	31.84
	Std	12.22	8.53	10.69	10.61
	Median	30.55	30.50	26	29
	Min/Max	21/65	21/59	21/61	21/65
	Q1/Q3	24.5/42	25.5/35.5	21/32	24/36
Day 8	N	28	27	28	83
	Missing	0	1	0	1
	Mean	32.36	31.15	29.29	30.93
	Std	10.95	8.61	10.56	10.07
	Median	29.50	29	26	29
	Min/Max	21/60	21/61	21/61	21/61
	Q1/Q3	24/36.5	25/36	22/31.5	23/35
Day 9	N	28	26	28	82
	Missing	0	2	0	2
	Mean	30.68	30.19	27.71	29.51

	Std	11.58	8.58	9.88	10.09
	Median	27	27	25	27
	Min/Max	21/60	21/61	21/59	21/61
	Q1/Q3	23.5/30.5	25/34	21.5/29	23/31
Day 10	N	28	28	28	84
	Missing	0	0	0	0
	Mean	29.61	29	27.11	28.57
	Std	11.61	8.35	10.01	10.01
	Median	25.5	27.50	24	25
	Min/Max	21/63	21/61	21/60	21/63
	Q1/Q3	23/30.5	22.5/32.5	21/28	22/30.5
Day 11	N	28	24	28	80
	Missing	0	4	0	4
	Mean	28.14	28.92	25.82	27.56
	Std	13.20	8.72	9.93	10.82
	Median	24	27	23	24
	Min/Max	1/62	20/61	21/61	1/62
	Q1/Q3	21/30.5	23/32	21/24.5	21/29.5

Table 5. Absolute change from baseline (day 1) of PCR test positivity by visit for both the ORF1a/b and the E gene (modified Intention-To-Treat analysis set)

	Azelastine 0.1% (A)		Azelastine 0.02% (B)		Placebo (C)	
	ORF1a/b	E	ORF1a/b	E	ORF1a/b	E
Day 2	1 (3.7 %)	1 (3.7 %)	-	-	1 (3.8 %)	1 (3.8 %)
Day 3	1 (3.7 %)	1 (3.7 %)	1 (3.6 %)	1 (3.6 %)	-	-
Day 4	1 (3.7 %)	1 (3.7 %)	1 (3.6 %)	-	-	-
Day 5	3 (11.1 %)	3 (11.1 %)	2 (7.1 %)	1 (3.6 %)	1 (3.8 %)	1 (3.8 %)
Day 8	6 (22.2 %)	3 (11.1 %)	8 (28.6 %)	5 (17.9 %)	-	-
Day 11	12 (44.4 %)	8 (29.6 %)	8 (28.6 %)	6 (21.4 %)	6 (23.1 %)	3 (11.5 %)

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Figures

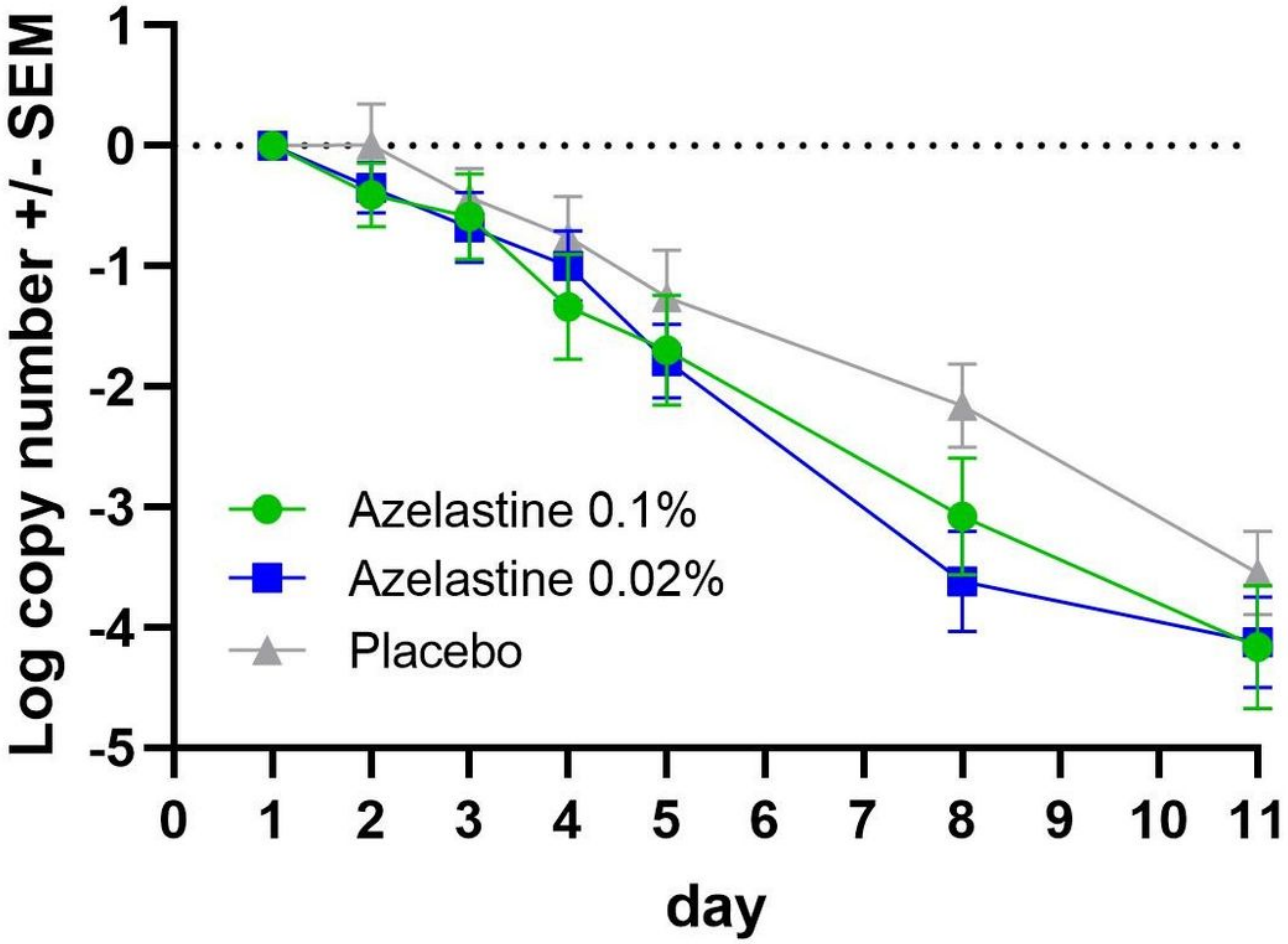

Figure 1

Absolute changes in viral copy numbers (log10 cp/ml) from baseline (day 1) over time (Modified intention-to-treat analysis set) based on the ORF 1a/b gene

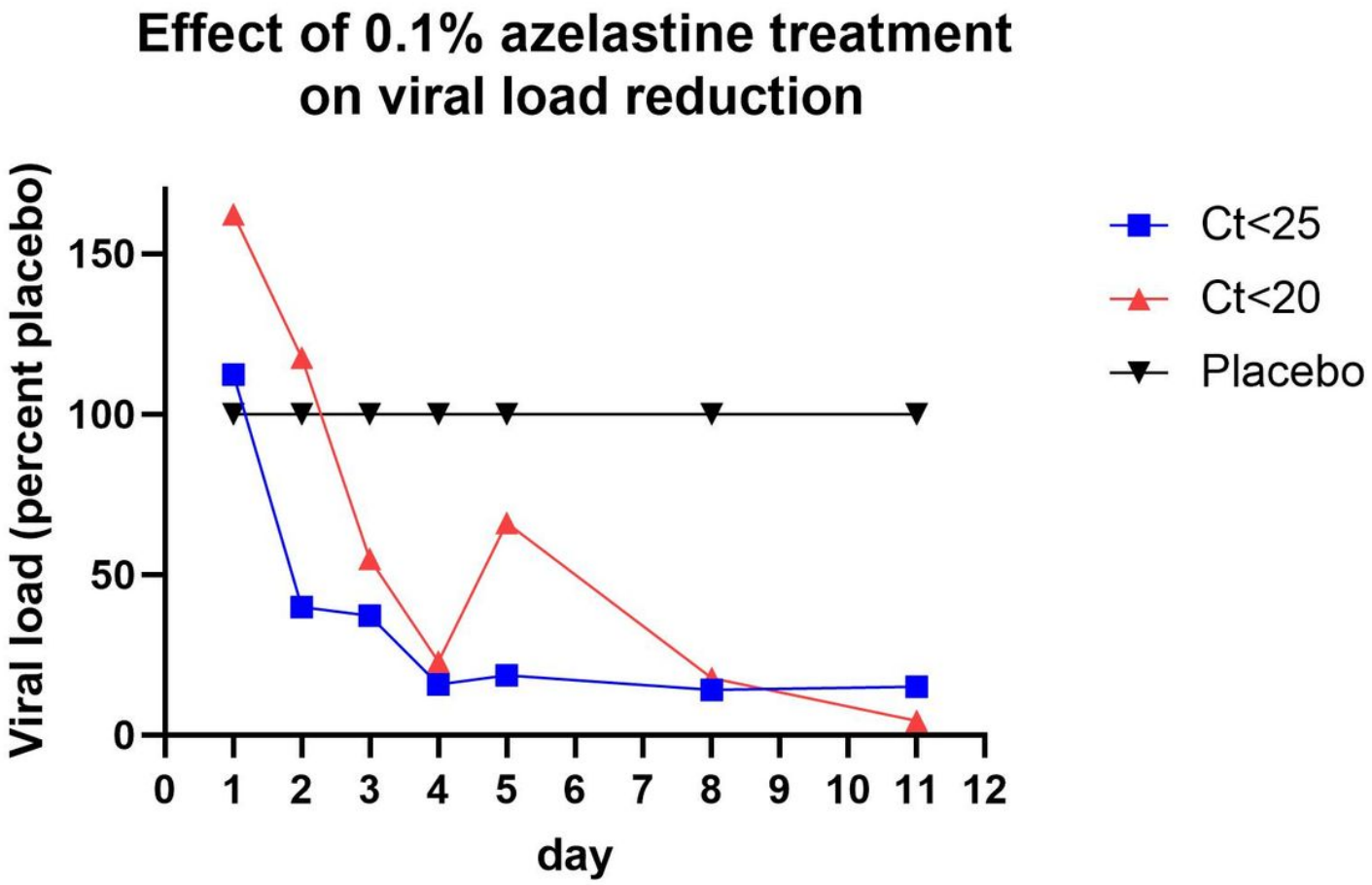

Figure 2

Viral load in the 0.1% azelastine nasal spray group relative to the viral load in the placebo group (normalized to 100%) over time for patients with baseline Ct values lower than 25 and lower than 20, based on the PCR analysis of the ORF1a/b gene. Number of patients in the groups: Ct<25: aze 0.1% n=19, placebo n=17; Ct<20: aze 0.1% n=10, placebo n=6.

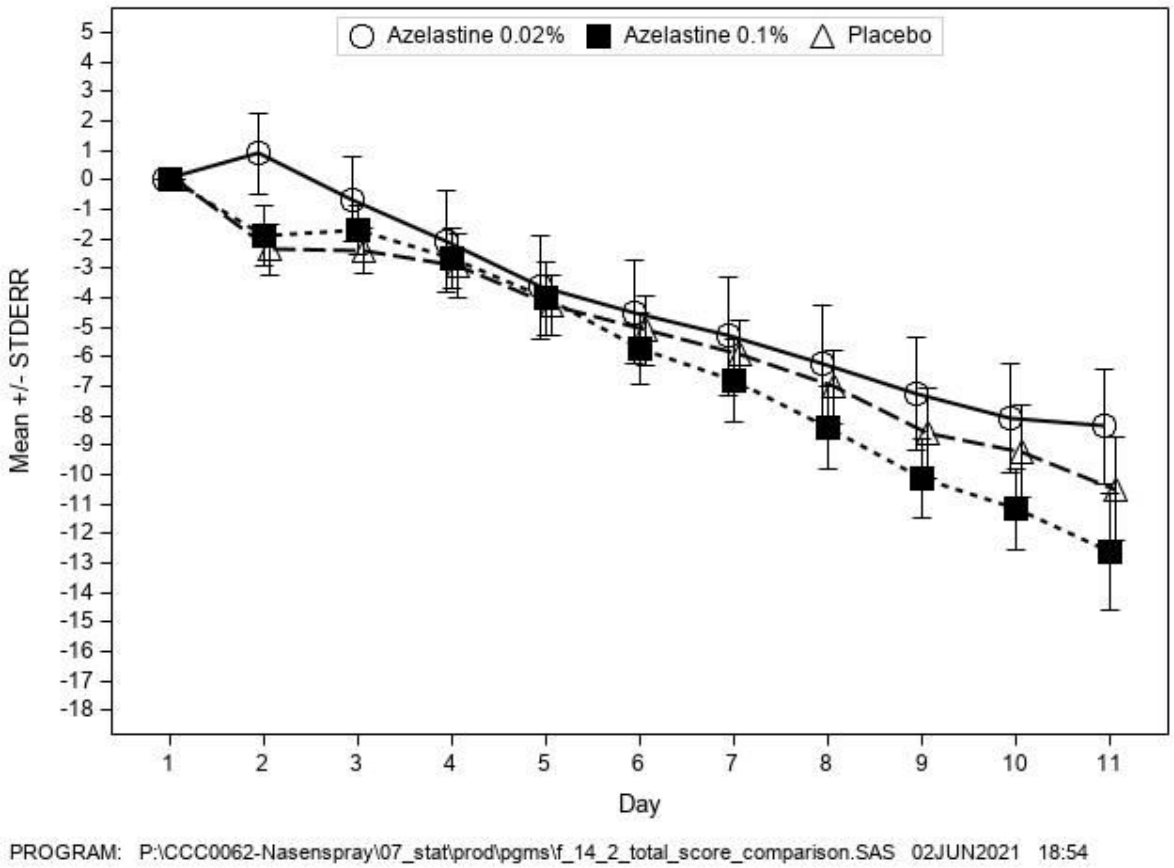

Figure 3

Absolute changes from baseline (day 1) of total symptom score over time (Modified Intention-to-treat analysis set).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- TableS2.docx
- TableS3.docx
- TableS4.docx
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