

1 CLINICAL TRIAL REPORT

2 Unger-Manhart et al

3 **Demonstration of a Decongestant Effect of “Coldamaris**
4 **Akut” Compared to Saline Nasal Spray in Participants**
5 **Suffering from Seasonal Allergic Rhinitis**

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26 Abstract

27 **Purpose:** Carrageenan-containing nasal sprays are known to alleviate symptoms of common cold
28 and allergic symptoms by building a barrier against airborne intruders. The objective of this study
29 was to develop a hyperosmolar nasal spray with barrier-forming properties and to demonstrate its
30 decongestant effect in the context of allergic rhinitis.

31 **Methods:** The efficacy of the nasal spray components was first demonstrated *in vitro* by a virus
32 replication inhibition, water absorption, and barrier assay. Clinical efficacy was assessed in a
33 randomized, controlled, crossover trial, where adults with a history of severe seasonal allergic
34 rhinitis were exposed to grass pollen allergens under controlled conditions for a total of 6 hours.
35 Participants received either the carrageenan- and sorbitol containing nasal spray (CS) or saline
36 solution (SS) after 1h45min of allergen exposure. After one week, participants repeated the
37 exposure, receiving the treatment (CS or SS) they had not received before. The primary efficacy
38 endpoint was the mean change in 'Nasal Congestion Symptom Score' (NCSS) during the allergen
39 exposure. Secondary efficacy endpoints were nasal airflow, nasal secretion, total nasal symptom
40 score (TNSS), total ocular symptom score (TOSS) and total respiratory symptom score (TRSS).

41 **Results:** Preclinical assays showed virus-blocking, barrier building and water withdrawing
42 properties of the CS components. In the clinical study, a total of 46 participants were screened, 41
43 were randomized and 39 completed the study. There was no significant difference in mean NCSS
44 change from pre- to post-treatment between CS and SS (mean difference of 0.02 [95% CI -0.19;
45 0.24] during the first 2 hours after treatment) when analyzed by intention-to-treat. However, nasal
46 airflow increased over time after treatment with CS, while it declined after SS, leading to a growing
47 difference in airflow between CS- and SS-treated participants ($p=0.039$ at 6:00h). The anterior
48 nasal airflow increased after treatment in 23/38 (61%) of the CS treated participants, compared to
49 only 13/38 (34%) of the SS treated participants ($p=0.024$). The mean nasal secretion over 2-6 h
50 was reduced by 1.00 g or -25% after CS ($p=0.003$) compared to pre-treatment, while it was reduced
51 by only -0.50 g after SS ($p=0.137$). No significant differences in TNSS, TOSS and TRSS were
52 observed between CS and SS treatments.

- 53 **Conclusion:** CS builds a barrier at the mucosa against viruses and dust and is safe and effective
54 in alleviating nasal congestion, nasal airflow and nasal secretion in adults with grass pollen allergy.
- 55 **Trial registration:** NCT04532762
- 56 **Keywords:** Allergic rhinitis, nonpharmacological, drug-free, barrier, carragelose, carrageenan

57 Introduction

58 Nasal congestion, also described as fullness, blockage, or obstruction of the nasal cavity, is a
59 frequently described symptom in clinical practice. It can significantly impair quality of life, reduce
60 daytime productivity at work or school, and negatively impact night-time sleep time and quality.¹

61 Nasal congestion is usually treated with local decongestants like Xylometazoline or Oxymetazoline.
62 Unfortunately, rebound swelling of the mucosa is observed upon prolonged use of these topical
63 vasoconstrictors. This often leads to a gradual overuse and a vicious circle of self-treatment, which
64 patients are often not aware of.^{2,3}

65 Nasal congestion is caused by air-borne irritants like tobacco smoke or dust, or by viruses and
66 allergens which cause viral and allergic rhinitis and sinusitis, respectively. Allergic rhinitis is a type
67 I allergic reaction where otherwise innocuous allergens such as pollen or animal dander crosslink
68 receptor bound IgE on mast cells.⁴ This crosslinking results in a biphasic response. The early phase
69 is characterized by the release of pre-formed mediators such as histamine which cause
70 characteristic symptoms like pruritus, rhinorrhea, sneezing, and nasal congestion. The late phase
71 is characterized by the release of newly synthesized mediators such as cytokines and chemokines.
72 The latter strongly contribute to inflammation and thereby to a worsening of the disease. Seasonal
73 allergic rhinitis or hay fever is caused by seasonal peaks in the airborne load of pollens and is the
74 most common type of allergic rhinitis. It is one of the most common chronic conditions in high-
75 income countries⁵ and it is estimated that in Europe, up to 40% of the population suffer from pollen
76 allergy.^{6,7} In contrast to viral rhinitis, which is usually self-limiting with symptom duration of about 1
77 to 2 weeks, symptoms of allergic rhinitis can continue over longer periods. Allergic patients using
78 topical decongestion are therefore at higher risk of the rebound effect and would benefit from a
79 decongestant that does not induce this habituation effect.

80 Marinomed Biotech AG has developed nasal sprays based on iota-carrageenan (Carragelose®), a
81 natural polymer from red seaweed, which forms a protective layer on mucosal surfaces that
82 prevents viruses and allergens from interacting with the mucosal surface. Carragelose® is certified
83 for marketing in the EU, parts of Asia and Australia, as a component of nasal sprays, throat sprays

84 and lozenges. Previous studies have shown that carrageenan-containing nasal sprays have a
85 broad, non-specific mode of action and prevent attachments of small particles like virus or pollen
86 to mucosal cells. This has been shown by us and others pre-clinically,⁸⁻¹⁰ and clinically.¹¹⁻¹⁷
87 Carrageenan-containing nasal sprays reduce the symptoms of common cold and the viral load in
88 nasal lavage.¹⁴ Symptom duration is shorter and viral titers in nasal fluids decrease faster in patients
89 of common cold when treated with carrageenan-containing nasal spray compared to placebo.^{12,13}
90 Since the virus-blocking effect of carrageenan is based on its physical barrier function, we
91 hypothesized that it can act also against other small particles like pollen, resulting in the alleviation
92 of AR symptoms.

93 To broaden the beneficial effect of our nasal spray, we wanted to add a decongestant activity by
94 enhancing the osmolarity of the solution. This causes outflux of water from the nasal mucosa cells,
95 thereby reducing mucosal swelling and hence nasal congestion. A hypertonic nasal spray
96 containing carrageenan combines decongestant and anti-viral activity. Hypertonicity could be
97 achieved by addition of ionic and/or non-ionic osmolarity givers like sodium chloride (NaCl).
98 However, carrageenans change their conformation depending on the ionic strength of the
99 environment.^{18,19} Enhancing osmolarity using NaCl might therefore affect their anti-viral properties.
100 Alternatively, hypertonicity could be achieved by adding sorbitol, a water-soluble, membrane
101 impermeant polyol (sugar alcohol) that is frequently used in food processing to preserve moisture
102 and add sweetness and texture.

103 Here, we report preclinical in vitro and ex vivo data that are the basis for optimization of the
104 decongestant nasal spray formulation. Furthermore, we show results of a randomized, controlled,
105 crossover clinical trial on a decongestant effect of the CS in adults with a history of severe seasonal
106 allergic rhinitis (SAR). The primary objective of this trial was to demonstrate a decongestant effect
107 on the nasal mucosa of the CS in comparison with 0.5% saline solution nasal spray (SS). The
108 secondary objective was to demonstrate the clinical performance of the CS in comparison with
109 saline solution as assessed by objective measurements of nasal airflow and nasal secretion as well
110 as patient-reported nasal, ocular and respiratory symptoms.

111 **Methods**

112 ***Preclinical assays***

113 **In vitro viral inhibition assay**

114 To test if osmolarity could be adjusted with NaCl without compromising the virus-blocking
115 effectiveness of carrageenan, a series of formulations containing 1.2 mg/ml iota-carrageenan and
116 0.4 mg/ml kappa-carrageenan with sodium chloride concentrations between 0.5% and 2.3% were
117 tested against Human rhinoviruses HRV1a and HRV8. Hela cells were seeded in 96-well plates. 4-
118 fold concentrated serial dilutions of the test sample (CS containing varying concentrations of NaCl)
119 and 4-fold concentrated virus dilution were prepared. Equal volumes of virus and test sample
120 dilutions were mixed and incubated at RT for 30 minutes. The mixture was diluted with an equal
121 volume of medium with 4% fetal bovine serum and antibiotic/antimycotic before it was added to the
122 cells for infection at a multiplicity of infection (MOI) of 0.7. After 48 hours at 33°C, cells were washed,
123 and viability was assessed with Alamar Blue staining. Viability was corrected for toxicity of
124 increasing salt concentrations and normalized to the viability of non-infected cells. The same
125 experimental set-up was used to test viral inhibition effectiveness of the final formulation of the
126 commercial product, containing 1.2 mg/ml iota-carrageenan, 0.4 mg/ml kappa-carrageenan, 0.5%
127 NaCl, and 7% sorbitol in citrate/phosphate buffer. Half-maximal inhibitory concentrations (IC₅₀)
128 were calculated with XLfit Excel add-in version 5.3.1. Results were normalized to toxicity and non-
129 infected control.

130 All percentages referring to nasal spray components here and in the following subsections are %
131 weight/volume.

132

133 **Hemagglutination assay**

134 This assay was applied to assess anti-viral activity against coronavirus hCoV OC43. On a 96-well
135 plate, two hemagglutination units of hCoV OC43 per well are incubated with a semi logarithmic

136 dilution series of test or control samples for 10 min at RT (final concentrations: 0.002-3µg/ml iota-
137 carrageenan diluted in 0.5% to 2.6% NaCl with or without 7% sorbitol and McIlvaine buffer). A
138 suspension of chicken red blood cells (1% v/v in PBS) is added to each well to allow
139 hemagglutination of RBCs by the virus for 1.5 hrs at 4°C. At the time point of assay evaluation,
140 control RBCs in the absence of carrageenan are fully agglutinated by the virus, whereas inhibition
141 of hemagglutination can be observed in samples treated with carrageenan up to a certain dilution
142 factor. The minimal inhibitory concentration of each sample is noted for comparison of the anti-viral
143 effectiveness of each sample under these assay conditions. As an internal control, a specific batch
144 of iota-carrageenan is used (assay reference).

145 **Ex vivo dehydration assay**

146 The swine nasal mucosa was received from “University Clinic for Swine” at the University of
147 Veterinarian Medicine Vienna. The nasal mucosa was excised from euthanized pigs and punched
148 out into equal circular pieces with a diameter of 10mm. The mucosa pieces were weighed and put,
149 the mucosa-site upward, into 48-well plates. 250 µl test solution was added to each well. Test
150 solutions were iota- and kappa-carrageenan with 0.5% NaCl and 7% sorbitol; iota- and kappa-
151 carrageenan with 0.5% NaCl without sorbitol; and a 2.4% NaCl solution. The plate was incubated
152 for 60 minutes at 37°C, after which the mucosa pieces were weighed again.

153 **In vitro barrier assay**

154 A 1.25% agar solution was filled into the wells of a 96-deep-well plate and was left to solidify o/n
155 at 4°C. 200 µl of CS and of negative control were added on top of the agar block. The negative
156 control sample contained sorbitol and NaCl in same concentration as in CS but did not contain the
157 barrier forming component carrageenan. Fluorescent beads of 0.3 µm or 1.0 µm, respectively, were
158 added and incubated for 3h at RT. Following multiple wash steps with 0.5% NaCl solution, beads
159 were extracted from agar blocks using 0.1% Tween20 in PBS o/n at 4°C with 900rpm shaking.
160 Extraction supernatants were transferred into a 96-well black flat bottom plate and analyzed in a

161 plate photometer with an excitation and emission wavelength of 485nm and 520nm, respectively.

162 Percent blocking was calculated relative to the amount of beads extracted from the negative control.

163 ***Clinical study***

164 **Study design**

165 This was a prospective, controlled, double-blinded randomized two-way cross-over single site study
166 in adult female and male participants with severe grass pollen induced seasonal allergic rhinitis
167 (SAR). The study evaluated two treatments, namely the carrageenan- and sorbitol containing nasal
168 spray (CS) and a saline solution (SS) nasal spray. The study was conducted at the Vienna
169 Challenge Chamber (VCC) in Vienna, Austria. The Ethics Committee of the City of Vienna oversaw
170 trial conduct and documentation. The study was designed to include 5 visits. At visit 1 (screening
171 visit), participants were screened for appropriate allergic response. At visit 2, which could be done
172 on the same day as visit 1, medical and allergic history and inclusion/exclusion criteria were
173 assessed and blood samples for safety lab were withdrawn. At visit 3, scheduled 7 days after visit
174 2, participants were randomized to one of the two treatment arms (CS or SS) in a fully blinded
175 fashion (details of randomization see below) and underwent their first six-hour allergen challenge
176 session. Approximately 1 hour and 45 minutes after start of allergen exposure, participants were
177 dosed with the treatment they had been randomized to, and continued exposure for a total of 6
178 hours. (first treatment block). At visit 4, scheduled 7 days after visit 3 to allow complete symptom
179 relief from the previous challenge, participants were exposed to the second allergen challenge
180 (second treatment block) and crossed over to the treatment that they had not received in the first
181 block. A follow-up visit (end of study visit, visit 5) was scheduled one week after the second
182 treatment block. Participants were asked to record AEs and the use of concomitant medications for
183 the entire duration of the trial.

184 **Participants**

185 Participants were female and male adults aged between 18 and 65 years of any ethnicity/race, with
186 a documented clinically relevant allergic history of moderate to severe SAR to grass pollen for the
187 previous two years. Participants were selected from the VCC database and had to satisfy all
188 inclusion and exclusion criteria to be enrolled into the study. Key inclusion criterion was a moderate
189 to severe response to standard grass pollen allergen mixture within the first 2 hours in the VCC,
190 defined as total nasal symptom score (TNSS) of at least 6 (out of 12) with the necessity to score at
191 least “moderate = 2” for the single symptom ‘nasal congestion’. TNSS is the sum of ‘nasal
192 congestion’, ‘rhinorrhea’, ‘itchy nose’ and ‘sneezing’, each scored on a categorical scale from 0 to
193 3. In addition, participants had to fulfill the following inclusion criteria: a positive Skin Prick Test
194 (SPT) response (wheal diameter at least 3 mm larger than diluent control) to grass pollen solutions
195 (standard Allergopharma) at screening or within the last 12 months prior to study start; positive
196 serum specific IgE against recombinant major allergen components of the used grass pollen e.g.,
197 g6 (specific CAP IgE ≥ 0.70 kU/L) at screening or within the last 12 months prior to study start; and
198 a forced expiratory volume in 1 second (FEV1) of at least 80% of reference value²⁰ at screening.
199 Asthma patients were allowed into the study only if the asthma condition was mild or intermittent,
200 and if not treated with steroids. Exclusion criteria comprised prior and ongoing conditions, diseases
201 and treatments that may interfere with the study intervention and outcomes. Female participants of
202 child-bearing potential were required to use birth control.

203 **Randomization and blinding**

204 Randomization numbers were allocated to the study participants in ascending order of their
205 Screening Numbers following their attendance at Visit 3 (first treatment block). They were
206 randomized using a cross-over randomization with balanced blocks. All personnel involved in the
207 study, including investigators, site personnel, and sponsor’s staff were blinded to the randomization
208 codes. Persons responsible for labeling of investigational products were un-blinded, but not
209 involved in other study activities. Un-blinding occurred at the end of the study.

210 **Interventions and procedures**

211 During each treatment period, participants were exposed to standard grass pollen allergen mixture
212 in the VCC for six hours using a validated method.^{21,22} During the challenge session, participants
213 were under constant supervision by, and could communicate with, medical staff outside the
214 chamber. The chamber was charged with 100% fresh air, which was conditioned (filtered, heated,
215 dried, cooled, and humidified) and then loaded with the challenge agent, a mixture of four grass
216 pollen species (Timothy, Orchard, Perennial rye and Sweet vernal grass) (Allergon SB, Sweden).
217 Air temperature (24°C), humidity (40%) and allergen load (1500 grains/m³) were constantly
218 monitored and maintained. During the 6 hours challenge, subjective nasal symptoms (nasal
219 congestion, rhinorrhea, itching, sneezing) as well as ocular and respiratory symptoms were
220 recorded every 15 minutes. Nasal airflow was measured by active anterior rhinomanometry (AAR)
221 at a pressure difference of 150 Pascal across the nasal passages (sum of the right and left nostril
222 values). Nasal airflow was evaluated immediately before and every 30 minutes during exposure,
223 with an additional assessment at timepoint 2h 15min. Nasal secretion was evaluated by weighing
224 paper tissues used by the participants during their stay in the chamber and collected every 30
225 minutes. 1h 45min after entering the challenge chamber, i.e., after developing pronounced allergic
226 nasal symptoms including nasal congestion, participants applied 1 puff per nostril of either CS or
227 SS. This resulted in a residual observation period of 4h 15min.
228 CS contained 1.2mg/ml iota-carrageenan, 0.4 mg/ml kappa-carrageenan, 7% (w/v) sorbitol, 0.5%
229 (w/v) sodium chloride, 1 mg/ml ethylene diamine tetra acetate, buffer and purified water. SS
230 contained 0.5% sodium chloride in water.

231 **Endpoints**

232 The primary efficacy endpoint was the mean difference between CS and SS of the 'Nasal
233 Congestion Symptom Score' (NCSS) measured every 15 min during allergen exposure. Secondary
234 efficacy endpoints were nasal airflow as assessed by active anterior rhinomanometry, total nasal
235 symptom score (TNSS; sum of the symptoms 'nasal congestion', 'rhinorrhea', 'itchy nose', and
236 'sneezing'), total ocular symptom score (TOSS; sum of the symptoms 'ocular itching', 'redness',

237 'watery eyes'), total respiratory symptom score (TRSS; sum of the symptoms 'cough', 'wheeze',
238 'dyspnea'), and nasal secretion. Each individual symptom of NCSS, TNSS, TOSS and TRSS was
239 rated on a scale from 0 to 3, whereas "0" corresponded to "no symptoms", "1" to "mild symptoms"
240 (easy to tolerate), "2" to "moderate symptoms" (bothersome, but tolerable) and "3" to "severe
241 symptoms" (hard to tolerate). Safety assessments included frequency and severity of AE, related
242 AE and serious AE (SAE) throughout the study. In addition, vital signs (blood pressure, pulse rate,
243 temperature and breathing frequency) were assessed at every visit, pre-and post-challenge. Lung
244 function was assessed at screening as well as before allergen challenge and every 2 hours during
245 the allergen challenge by measuring the Forced Expiratory Volume in 1 second (FEV₁) using a
246 Piston Spirometer. Physical examination, laboratory blood analysis and ECG were conducted at
247 screening and at the follow-up visit.

248 **Statistical analysis**

249 Sample size calculation was based on the expectation of a mean difference of 0.6 points with
250 standard deviation of 1.1 (SS = 2, CS = 1.4, effect size $d=0.56$ and a power = 90%) which was
251 derived from previous studies. Thus, $n=36$ participants were needed at an alpha level of $p=0.05$.
252 Considering the dropout rate of 10-15%, up to 50 participants needed to be screened to randomize
253 about 42 participants in order to get evaluable data from at least 36 participants.

254 Safety analyses including vital signs, laboratory data and AEs, were carried out in the safety
255 population defined as all participants starting the challenge provocation qualification session.

256 Efficacy was analyzed in the Full Analysis Set (FAS) and in the Per-Protocol Set (PPS). The FAS
257 comprised all participants who were randomized and was analyzed following the intent-to-treat
258 (ITT) principle, according to the treatment they have been assigned at randomization. The PPS
259 comprises all participants in the FAS who did not have any clinically important protocol deviation.

260 The primary efficacy variable was analyzed in a confirmatory way between the two conditions CS
261 and SS, assuming superiority for CS versus SS. The null hypothesis was defined as:

262 *Mean NCSS [Delta pre-treatment (1:45h) - post-treatment (mean 2-4h)] {CS} ≤ Mean NCSS [Delta*
263 *pre-treatment (1:45h) - post-treatment (mean 2-4h)] {SS}*

264 The alternative hypothesis was formally defined as:

265 $Mean\ NCSS\ [Delta\ pre-treatment\ (1:45h) - post-treatment\ (mean\ 2-4h)]\ \{CS\} > Mean\ NCSS\ [Delta$
266 $pre-treatment\ (1:45h) - post-treatment\ (mean\ 2-4h)]\ \{SS\}$

267 A 95% confidence interval for the mean difference of the two treatments was calculated. The
268 superiority comparison of CS versus SS was performed using analysis of variance (ANOVA)
269 appropriate for the cross-over design. Period (first or second treatment block) was included in the
270 analysis model as a fixed effect to confirm the assumption of no period effect. Participant was
271 included in the model as a random effect. Superiority was to be postulated if the lower bound of the
272 95% confidence interval was >0 .

273 Secondary efficacy variables were analyzed in an explorative sense and are presented using
274 descriptive methods. Exploratory efficacy analysis was performed for mean differences between
275 the two treatments for consecutive intervals from 2h onward to 6h analogous to the primary efficacy
276 analysis. Respective statistical tests and p-values are to be regarded as descriptive and not as
277 tests of hypotheses.

278 All attempts were made to collect all data per protocol. Missing or invalid data was neither replaced
279 nor extrapolated. Outliers were not excluded from the primary analyses. Significance level was set
280 to $\alpha=5\%$. R version 4.0.3 was used for all statistical analyses.

281 Results

282 *Results Part 1: Preclinical Development*

283 Carrageenan containing nasal sprays are used to prevent and treat viral infections of the respiratory
284 tract by blocking the viruses' attachment to the mucosa. To enhance the benefit and broaden the
285 applicability of the barrier-forming nasal spray, a decongestant effect should be added to the
286 formulation. Usually, intranasally applied hyperosmotic saline solutions are used to withdraw water
287 from the nasal mucosa, thereby reducing intranasal swelling. However, we found that increasing
288 salt concentrations reduced the carrageenan's capacity to block the attachment of human
289 rhinovirus and of human coronavirus to cells. As shown by IC₅₀ values in **Table 1**, increasing sodium
290 chloride concentrations reduced the virus-blocking capacity of the carrageenan against human
291 rhinovirus HRV1 and HRV8 as well as against Coronavirus hCoV OC43 in a dose-dependent
292 manner. Therefore, the formulation was adjusted to 0.5% sodium chloride to preserve the
293 carrageenan's beneficial virus-blocking effect. To achieve hyperosmotic activity, sorbitol was added
294 to the formulation at a concentration of 7%, which increased the formulation's osmolality, but in
295 contrast to high concentrations of sodium chloride, preserved the virus-blocking activity of
296 carrageenan (also shown in **Table 1**).

297 After confirming that addition of buffer did not influence the antiviral activity of carrageenan (data
298 not shown), the final product was formulated with 1.2 mg/ml iota-carrageenan, 0.4 mg/ml kappa-
299 carrageenan, 0.5% NaCl, 7% sorbitol in citrate/phosphate buffer with an osmolality of 787
300 mosmol/kg, corresponding to the osmolality of hyperosmolar saline solutions with concentrations
301 of 2.3-3%. This formulation was then used for ex vivo experiments as well as for the clinical study.
302 Ex vivo experiments showed that incubation of nasal porcine mucosa with CS or a 2.4% saline
303 solution of similar osmolality withdrew considerable amounts of liquid from the mucosa, resulting in
304 a weight loss of 21±5% and 14±8%, respectively. In comparison, the weight of the mucosa
305 incubated with carrageenan in 0.5% NaCl remained equal (weight change of 1±6%), indicating that
306 the hyperosmolality alone, and not the carrageenan, is responsible for the weight loss (**Figure 1**).

307 These results demonstrate a beneficial effect of sorbitol when added to the CS that could support
308 nasal decongestion via its water draining properties.

309 A proof of principle for the barrier function of carrageenan in the formulation containing 7% sorbitol
310 and 0.5% NaCl was demonstrated by an in vitro barrier assay. This assay tests the ability of a
311 sample solution to inhibit diffusion of fluorescent beads, serving as surrogate for particulate matter,
312 into an agar block. As shown in **Figure 2**, CS nasal spray exhibited a blocking activity of $99\pm 0\%$ for
313 beads of $0.3\ \mu\text{m}$ diameter, and of $80\pm 2\%$ for beads of $1.0\ \mu\text{m}$ diameter. This means that the
314 protective layer formed by carrageenan allowed only 1% and 20%, respectively, of beads to reach
315 the agar block, compared to the negative control. This indicates that the nasal spray can provide
316 protection against external particles that might trigger or worsen allergic reactions.

317 **Results Part 2: Clinical Study**

318 The potential of the CS to treat nasal congestion in humans was examined in a clinical study in
319 patients with allergic rhinitis. **Figure 3, Panel A** gives a graphical overview of the study, **Panel B**
320 depicts the assessment carried out during each treatment block. Between September and October
321 2020, a total of 46 participants were screened after giving informed consent and were included in
322 the safety population. Of these, 41 participants fulfilled all in/exclusion criteria, were randomized to
323 one of the two possible treatment sequences, and hence constitute the FAS. 2 participants
324 discontinued, and 4 participants did not respond to either treatment with CS or SS and were thus
325 excluded from the per PPS based on the finding that hypertonic saline nasal spray has no effect
326 on nasal congestion in approximately 30% of the population.²³ No other exclusionary protocol
327 deviations were reported. **Figure 4** shows the flow of participants through the study.

328 Demographic characteristics are summarized in **Table 2**. 27/46 (59%) of the participants were
329 females, 19/46 (41%) were males. Participants were aged between 21 and 62 years, with a mean
330 age of 34.6 years (SD 10.9). The mean BMI was $23.9\ \text{kg/m}^2$, all participants' BMIs were below 30,
331 i.e., none of the participants was obese. All participants had a history of moderate to severe
332 seasonal allergic rhinitis (SAR) to grass pollen with a prior duration of between 8 and 43 years, on
333 average 23.5 years.

334 In the following, all efficacy results are shown for the FAS, analyzed by ITT. Results for the PP were
335 similar as for the FAS.

336 All participants developed nasal congestion upon the start of the allergen challenge. The mean
337 NCSS increased notably already after 15 min, further increased until timepoint 1h 45min, and was
338 reduced upon intake of either CS or SS (**Figure 5A**). The overall mean NCSS was 0.1 (SD 0.3)
339 before starting the allergen challenge (timepoint 00:00) and it increased to 2.3 (SD 0.7) after CS
340 treatment group and 2.2 (SD 0.5) after saline solution treatment at timepoint 1h45min
341 (**Supplementary Table S1**). However, only a small difference of 0.16 (SD 0.50) for CS and 0.11
342 (SD 0.53) for SS between pre-treatment NCSS (timepoint 1h45min, i.e., directly before the
343 treatment), and the mean NCSS across the time interval 2-4h could be detected (**Supplementary**
344 **Table S2**). No phase-effect (p -value >0.05 , Wilcoxon test) and no carry-over effect (p -value >0.05 ,
345 ANOVA) was observed. The mean difference between CS [Pre-treatment - \varnothing (2-4h)] and SS [Pre-
346 treatment - \varnothing (2-4h)] across all participants was 0.02, 95% CI [-0.19;0.24], $p >0.05$ (paired t-test)
347 (**Figure 5B**). With the lower bound of the 95% confidence interval <0 , superiority of CS versus SS
348 in terms of NCSS could not be established.

349 **Figure 6** shows the absolute nasal airflow in both treatments before treatment (timepoint 1h45min)
350 and at the end of the allergen challenge period. In total, an increased anterior nasal airflow was
351 measured in 23/38 (61%) of the participants after treatment with the CS, but in only 13/38
352 participants (34%) after SS treatment (**Table 3**). This difference between treatments was
353 statistically significant ($p=0.024$, McNemar's test for paired nominal data).

354 In order to unravel the temporal dynamics that led to the post-treatment differences, we also
355 followed nasal airflow changes over time by subtracting the mean pre-treatment value (timepoint
356 1h30min) from the mean post-treatment value of varying post-treatment periods (mean over 2-6h,
357 2:15-6h, 2:30-6h etc.). Positive values indicate higher nasal airflow post-treatment compared to
358 pre-treatment. As shown in **Supplementary Figure S1**, treatment with the CS led to an increase
359 of nasal airflow over the course of the 4 hours residual observation time compared to pre-treatment,
360 while it declined in the SS group. This led to a significantly higher airflow in the CS group compared
361 to the SS group at the end of the 6 hours treatment block: The difference between CS and SS in

362 nasal airflow change from pre-treatment to the end of the 6h treatment block in the FAS (ITT)
363 population was 54.29 ml/s (95% CI 2.92; 105.66). The difference was significantly in favor of the
364 CS (p=0.04, paired t-test) (**Supplementary Table S3**).

365 Changes in nasal secretion from pre- to post-treatment were calculated in an analogous manner.
366 In both groups, nasal secretion declined post-treatment when compared to pre-treatment. The
367 difference in nasal secretion from pre- to post-treatment was more pronounced in the CS group
368 than in the SS group (**Figure 7**). For the CS, the weight of nasal secretion changed from 3.99 g at
369 pre-treatment to 2.99 g averaged over the entire residual observation time (2-6h), representing a
370 mean tissue weight difference of -1.00 g or -25% (p=0.003, t-Test). After SS, the mean tissue weight
371 difference from pre-treatment to 2-6h, was only -0.50 g (p=0.137, Wilcoxon signed rank test). These
372 results indicate that nasal secretion declined more strongly after CS than after SS treatment (**Table**
373 **4 and Figure 7**).

374 TNSS, TOSS and TRSS over the 6 hours treatment block did not show any pronounced differences
375 between CS and S group (data not shown).

376 In the safety population, a total of 3 adverse events occurred in 2 participants during the trial:
377 pyrexia (mild), nasopharyngitis (moderate) and pharyngitis (severe) (**Table 5**). Pharyngitis and
378 pyrexia occurred in the same participants 4 days after the first treatment block with SS.
379 Nasopharyngitis occurred 4 days after the first treatment block with CS. None of them was
380 considered related to the study treatment, none was serious, all were resolved by study end. Both
381 participants missed the second treatment block and terminated the trial prematurely due to these
382 AEs.

383 All vital signs and laboratory values showed no particular differences between baseline and follow-
384 up visit (data not shown), indicating good tolerability of both allergen challenge and treatment with
385 CS and saline solution.

386 Discussion

387 This paper includes preclinical and clinical data demonstrating the safety and efficacy of a
388 carrageenan- and sorbitol -containing (CS) nasal spray. The in vitro/ex vivo data indicate that the
389 formulation is osmotically active while preserving the barrier-forming, virus-blocking capacity of the
390 carrageenan. The clinical data show that the CS nasal spray is safe and well tolerable in
391 participants with moderate to severe SAR. Although the primary endpoint based on the subjective
392 rating of nasal congestion was not met, two objective parameters, nasal airflow and nasal secretion,
393 showed a significant improvement upon treatment with CS nasal spray. Nasal airflow increased
394 upon CS administration, but decreased upon administration of saline solution, leading to a
395 significantly higher airflow in CS treated participants at the end of the challenge. The majority (60%)
396 of participants had an increased nasal airflow after CS, but only 34% had an increased nasal airflow
397 after SS administration. The amount of nasal secretion was reduced both after CS and SS
398 administration, but this reduction was significant only after the CS. The low incidence of adverse
399 events, none of them considered treatment-related, suggested safety of CS nasal spray similar to
400 saline solution used in this study and similar to carrageenan-only (no sorbitol) nasal spray as
401 demonstrated in previous studies.^{11-14,16,24,25}

402 The beneficial effect of the CS nasal spray is presumable achieved via multiple modes of action
403 attributed to carrageenan and sorbitol. First, carrageenan has excellent mucoadhesive properties
404 that are e.g. exploited for intranasal drug delivery.²⁶ We hypothesize that a mucoadhesive layer of
405 carrageenan forms a protective barrier in the nasal mucosa that prevents small particles like pollen
406 and dust to enter the nasal mucosa and hinders further induction or aggravation of AR symptoms
407 like nasal congestion and nasal secretion.¹⁷

408 Secondly, polyols like sorbitol are known and widely used as humectants in the cosmetics and food
409 industry based on their hygroscopic properties.²⁷ In the context of rhinitis, xylitol, another polyol
410 with similar properties as sorbitol, was shown to keep the nasal passages and sinuses moist and
411 clean for a longer time than saline alone. 5-days-use of a hyperosmolar xylitol-containing nasal
412 spray led to significant improvement of the overall quality of life score compared to pre-treatment

413 in participants suffering from nasal obstruction.²⁸ Moreover, a xylitol solution was as effective in the
414 treatment of rhinitis medicamentosa in rats as the glucocorticoid mometasone in the reversal of
415 histopathological changes caused by long-term treatment with oxymetazoline.²⁹

416 Strengths of this study include the cross-over design, in which each participant serves as their own
417 control, the random assignment to minimize possible effects from the order of treatments, and the
418 blinding of investigators, site personnel, and the sponsor's staff. Another strength is the use of an
419 environmental challenge chamber to induce AR symptoms, which allows to control environmental
420 conditions like temperature, humidity, and allergen type and concentration, and thus enables the
421 performance of allergology studies out of allergy season and under uniform allergen exposure
422 conditions. This limits variation and helps reducing the number of study participants. Moreover, use
423 of the challenge chamber allows the study personnel to supervise administration of medication and
424 documentation of outcomes, thereby enhancing participant compliance.³⁰⁻³⁶

425 The study has several limitations. One of them is the selection of the NCSS, a subjective scoring
426 scale, as primary endpoint. The rationale for the selection of the primary endpoint was that nasal
427 congestion comes with a significant impact upon patients' QOL, which is considered an important
428 determinant of the severity of nasal diseases.^{37,38} In fact, the degree of health-related QOL
429 impairment has been demonstrated to drive patients' choice between treatment options.³⁹

430 Assessment of QOL in the form of patient reported outcome measures (PROMs) is regarded a
431 standard outcome measures in clinical trials, acknowledging the fact that the classical, objective
432 outcome variables may only partially characterize the disease of the patient. However, the focus
433 on a PROM as primary endpoint also poses problems due to the low degree of correlation between
434 subjective and objective outcomes assessing nasal symptoms, as systematically reviewed by Ta
435 et al.⁴⁰ The authors consequently recommend to use objective outcome measures to complement
436 and confirm validated patient reported outcomes.⁴⁰

437 The findings of our study support this conclusion, showing discrepancies between subjective and
438 objective evaluations. As described in the results section, only very slight differences between
439 groups and between timepoints were observed by NCSS that may possibly reach significance only
440 with a much larger sample size. In contrast, differences between CS and SS in nasal airflow

441 improvement measured by AAR became significant towards the end of the allergen challenge,
442 indicating that this sensitive method is able to pick up subtle changes that cannot possibly be
443 detected by PROMs like the NCSS with the available number of participants. Rhinomanometry
444 enables the objective and accurate measurement of nasal congestion, and is considered the gold
445 standard for measuring nasal airway patency and resistance.⁴¹ The method has been
446 demonstrated to be sensitive in quantifying nasal patency after nasal provocation testing and to
447 assess the efficacy of medications used to treat nasal congestion/obstruction.⁴² The
448 implementation of rhinomanometry as objective endpoint in addition to the subjective symptom
449 scores is therefore a particular upside of this study. Analogously, objective determination of nasal
450 secretion revealed a significant reduction of nasal secretion after treatment compared to pre-
451 treatment, which was not captured by the TNSS with sufficient sensitivity.

452 In this study, we used the time window from 2 to 4h after start of allergen exposure, that is, starting
453 15min after treatment administration and ending 2h15min after treatment administration. This
454 interval was selected based on the expectation that the most pronounced effect of the treatment
455 would manifest shortly after treatment. The mean residence time of carrageenan at the mucosa of
456 approximately four hours was determined in a prior study using nasal mucociliary clearance (NMC)
457 time assessment in healthy volunteers,¹⁵ and we expected the most pronounced effect to manifest
458 in the first half of this period. However, nasal airflow continuously increased from post-treatment
459 until the end of the allergen challenge period.

460 In sum, based on our findings, we propose the CS as safe and effective treatment of mild to
461 moderate AR.

462 **Conclusion**

463 Coldamaris akut, a carrageenan- and sorbitol containing nasal spray, is considered safe and
464 effective in the relief of nasal symptoms in adults with grass pollen allergy.

465 **Ethics Statement**

466 The study was conducted in Austria in accordance with in accordance with the Declaration of
467 Helsinki on Ethical Principles for Medical Research Involving Human Subjects, the International
468 Council for Harmonisation Guideline on Good Clinical Practice, and all applicable local regulatory
469 requirements and laws. The study was approved by the Ethics Committee of the City of Vienna
470 (protocol code COA_19_03, EK 19/277/1219). Informed consent was obtained from all study
471 participants.

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474 **Disclosure**

475 NU, MM, HD and EP are employees of Marinomed Biotech AG. MS received consulting fees from
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477 This manuscript is also available at medRxiv preprint server.

478

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605

606 **Tables**

607 **Table 1: In vitro data: Virus-blocking effectiveness against HRV1a, HRV8 and hCoV OC43.**

608 Minimal inhibitory concentration of the various formulations determined in a virus inhibition assay

609 (for HRV1 and HRV8) or a hemagglutination inhibition assay (for hCoV OC43).

610

Effectiveness of various formulations	IC ₅₀ [µg/ml]	IC ₅₀ 95% CI [µg/ml]
HRV1a		
Carrageenan + 0.5% NaCl	1.8	0.7; 3.0
Carrageenan + 0.9% NaCl	5.6	4.0; 7.1
Carrageenan + 2% NaCl	26.5	23.0; 30.0
Carrageenan + 2.3% NaCl	40.7	35.0; 46.6
Fold Change (Carrageenan + 0.5% NaCl) vs. (Carrageenan + 2.3% NaCl)	22.3	
Carrageenan + 0.5% NaCl + 7% Sorbitol	3,7	2.2; 5.3
Carrageenan + 2.3% NaCl	104,5	82.8; 126.2
Fold Change (Carrageenan + 0.5% NaCl + 7% Sorbitol) vs. (Carrageenan + 2.3% NaCl)	27.9	
HRV8		
Carrageenan + 0.5% NaCl	2.3	1.0; 3.6
Carrageenan + 0.9% NaCl	4.1	2.9; 5.3
Carrageenan + 2% NaCl	8.1	5.5; 10.7
Carrageenan + 2.3% NaCl	15.6	8.8; 22.4
Fold Change (Carrageenan + 0.5% NaCl) vs. (Carrageenan + 2.3% NaCl)	6.9	
Carrageenan + Buffer + 0.5% NaCl + Sorbitol	0.8	0.7; 1.0
Carrageenan + 2.3% NaCl	2.3	1.5; 3.1
Fold Change (Carrageenan + 0.5% NaCl + 7% Sorbitol) vs. (Carrageenan + 2.3% NaCl)	2.9	
hCoV OC43		

Carrageenan + 0.5% NaCl	0.007	n.a.
Carrageenan + 0.9% NaCl	0.007	n.a.
Carrageenan + 2.0% NaCl	0.080	n.a.
Carrageenan + 2.3% NaCl	0.080	n.a.
Carrageenan + 0.5% NaCl + 7% sorbitol	0.007	n.a.
Fold Change (Carrageenan + 0.5% NaCl) vs. (Carrageenan + 2.3% NaCl)	11.4	
Fold Change (Carrageenan + 0.5% NaCl + 7% Sorbitol) vs. (Carrageenan + 2.3% NaCl)	11.4	

611

612 Note: Bold borders mark individual experiments.

613 Abbreviations: IC₅₀, inhibitory concentration neutralizing 50% of the virus; CI, confidence interval; NaCl, sodium chloride;

614 Carrageenan, 1.2 mg/ml iota-carrageenan and 0.4 mg/ml iota-carrageenan; n.a., not applicable.

615

616

Table 2: Clinical data: Demographic characteristics at baseline (Safety Population)

		617 All 618 participants (N=46) 619 620
Sex		621
Female	n (%)	622 27 (59%) 623
Male	n (%)	19 (41%) 624
Ethnicity		625 626
Caucasian	n (%)	28 (61%) 627
Not specified	n (%)	18 (39%)
Age	Years (min/max)	34.6 (21/62)
BMI	kg/m ² (min/max)	23.9 (19.1/29.8)

Abbreviations: BMI, body mass index.

Table 3: Clinical data: Improvement/worsening of airflow after 6h compared to pre-treatment (1h30min), evaluated within treatment groups for the FAS.

628

		CS (360 min - 90 min)	
		better or equal	worse
SS (360 min - 90 min)	better or equal	10	3
	worse	13	12

629

630

Abbreviations: CS, carrageenan-sorbitol containing nasal spray. SS, saline solution.

631

p-Value: 0.024 (McNemar's test for paired nominal data for comparison between treatments)

632

633 **Table 4: Clinical data: Tissue weight differences between pre-treatment [90 min] and the**
634 **mean of all post-treatment timepoints [120-360 min] for the FAS.**

635

636

Treatment	Mean Weight [g] ± SD			p-Value
	Pre-treatment	After treatment	Difference after - pre	
CS	3.99 ± 3.24	2.99 ± 2.16	-1.00 ± 1.96	0.003*
SS	3.07 ± 2.59	2.57 ± 1.87	-0.50 ± 1.70	0.137**

637

638 Abbreviations: CS, carrageenan-sorbitol containing nasal spray; SS, saline solution; SD, standard deviation.

639 Pre-treatment = mean at timepoint 90min

640 After treatment = mean of all timepoints from 2h to 6h

641 * t-test (Normality assumption confirmed)

642 ** Wilcoxon signed rank test (Normality assumption rejected)

643

644 **Table 5: Clinical data: Adverse events by SOC/PT and severity for the Safety Population**
645 **(N=46).**

646

SOC	PT	Mild	Moderate	Severe	Total
General disorders and administration site conditions		1	0	0	1
	Pyrexia	1	0	0	1
Infections and infestations		0	1	1	2
	Nasopharyngitis	0	1	0	1
	Pharyngitis	0	0	1	1

Abbreviations: SOC, system organ class; PT, preferred term.

647

648 **Supplementary Tables**

649 **Table S1: Clinical data: Nasal congestion symptom score (NCSS) of all time points for the**
 650 **FAS. N=40 for all timepoints.**

651

Time	Carrageenan-Sorbitol (CS) nasal spray						Saline solution (SS)					
	Mean	SD	LQ	UQ	Min	Max	Mean	SD	LQ	UQ	Min	Max
00:00	0.1	0.3	0.0	0.0	0	1	0.1	0.3	0.0	0.0	0	1
00:15	0.8	0.5	0.0	1.0	0	2	0.7	0.6	0.0	1.0	0	2
00:30	1.4	0.6	1.0	2.0	0	3	1.3	0.6	1.0	2.0	0	2
00:45	1.7	0.7	1.0	2.0	0	3	1.6	0.6	1.0	2.0	1	3
01:00	2.0	0.6	2.0	2.0	1	3	1.9	0.6	2.0	2.0	1	3
01:15	2.0	0.7	2.0	2.0	0	3	1.9	0.7	1.0	2.0	1	3
01:30	2.2	0.6	2.0	3.0	1	3	2.0	0.6	2.0	2.0	1	3
01:45	2.3	0.7	2.0	3.0	1	3	2.2	0.5	2.0	2.0	1	3
02:00	2.1	0.7	2.0	2.2	1	3	1.9	0.6	1.8	2.0	1	3
02:15	2.2	0.7	2.0	3.0	1	3	2.0	0.6	2.0	2.0	1	3
02:30	2.1	0.6	2.0	3.0	1	3	2.0	0.7	2.0	2.2	1	3
02:45	2.1	0.7	2.0	3.0	1	3	2.0	0.7	1.8	3.0	1	3
03:00	2.2	0.6	2.0	3.0	1	3	2.0	0.7	2.0	2.2	1	3
03:15	2.1	0.7	2.0	3.0	1	3	2.0	0.7	2.0	3.0	1	3
03:30	2.2	0.7	2.0	3.0	1	3	2.1	0.7	2.0	3.0	1	3
03:45	2.2	0.7	2.0	3.0	1	3	2.1	0.7	2.0	3.0	1	3
04:00	2.2	0.8	2.0	3.0	0	3	2.2	0.7	2.0	3.0	1	3
04:15	2.1	0.8	2.0	3.0	0	3	2.1	0.8	1.8	3.0	0	3
04:30	2.2	0.8	2.0	3.0	0	3	2.1	0.7	2.0	3.0	1	3
04:45	2.2	0.7	2.0	3.0	1	3	2.2	0.7	2.0	3.0	1	3
05:00	2.2	0.7	2.0	3.0	1	3	2.2	0.7	2.0	3.0	1	3
05:15	2.4	0.7	2.0	3.0	1	3	2.2	0.7	2.0	3.0	1	3
05:30	2.2	0.8	2.0	3.0	1	3	2.2	0.7	2.0	3.0	1	3
05:45	2.3	0.7	2.0	3.0	1	3	2.2	0.7	2.0	3.0	1	3
06:00	2.3	0.8	2.0	3.0	1	3	2.2	0.7	2.0	3.0	1	3

652

653

654

Abbreviations: CS, carrageenan-sorbitol containing nasal spray. SS, saline solution; SD, standard deviation; LQ, lower quartile; UQ, upper quartile.

655 **Table S2: Clinical data: Mean difference in NCSS [Pre-treatment - \varnothing (2-4h)] for the FAS.**

656

	CS	SS	CS – SS
Mean	0.16	0.11	0.02
SD	0.50	0.53	0.66
Median	0.00	0.00	0.11
Min	-0.89	-0.78	-1.56
Max	1.44	1.56	1.33
N	40 ¹	40 ¹	39 ²

657

658 Abbreviations: NCSS, Nasal Congestion Symptom Score. CS, carrageenan-sorbitol containing nasal spray. SS, saline
659 solution.

660

SD, standard deviation. Min, minimum value. Max, maximum value.

661

1 n = 40 out of 41 patients completed each treatment period

662

2 n = 39 out of 41 patients completed both treatment periods

663

664 **Table S3: Clinical data: Mean difference in AAR change from pre- to post-treatment**
 665 **between CS and SS for the FAS.** Values for the respective treatment period are first calculated
 666 individually by subtracting the mean pre-treatment airflow from the mean nasal airflow over the
 667 indicated post-treatment time period. Differences between treatments are computed likewise by
 668 subtracting the [mean pre- to post-treatment difference for SS] from the [mean pre- to post-
 669 treatment difference for CS]. Paired t-tests were applied to those differences. Differences above 0
 670 are favorable for the CS treatment.
 671

Observation interval	Mean difference between CS and SS	95% CI	P-Value ²
ø(2:00-6:00 h) - Pre-treatment	8.05	-24.05; 40.14	0.61
ø(2:15-6:00 h)) - Pre-treatment	12.23	-21.80; 46.27	0.47
ø(2:30-6:00 h)) - Pre-treatment	11.96	-22.55; 46.47	0.49
ø(3:00-6:00 h)) - Pre-treatment	17.07	-17.02; 51.16	0.32
ø(3:30-6:00 h)) - Pre-treatment	19.39	-14.34; 53.13	0.25
ø(4:00-6:00 h)) - Pre-treatment	23.34	-10.92; 57.61	0.18
ø(4:30-6:00 h)) - Pre-treatment	25.59	-10.87; 62.04	0.16
ø(5:00-6:00 h)) - Pre-treatment	28.23	-12.68; 69.14	0.17
ø(5:30-6:00 h)) - Pre-treatment	47.96	5.14; 90.78	0.03
ø(6:00 h)) - Pre-treatment	54.29	2.92; 105.66	0.04

672
 673 Abbreviations: CS, carrageenan-sorbitol containing nasal spray. SS, saline solution. CI, confidence interval.
 674 ¹ Mean Nasal Airflow, measured by active anterior rhinomanometry (AAR).
 675 ² Paired t-test
 676
 677

Figure 1: Ex vivo assay: Hyperosmolar effect of CS nasal spray with and without sorbitol. Weight decrease of ex-vivo porcine nasal mucosa after incubation for 60 minutes at 37°C in CS (carrageenan + 0.5% NaCl + 7% sorbitol in buffered aqueous solution), a 2.4% sodium chloride solution, or carrageenan + 0.5% NaCl in buffered aqueous solution without sorbitol (CS w/o sorbitol). Error bars represent standard deviation of replicates.

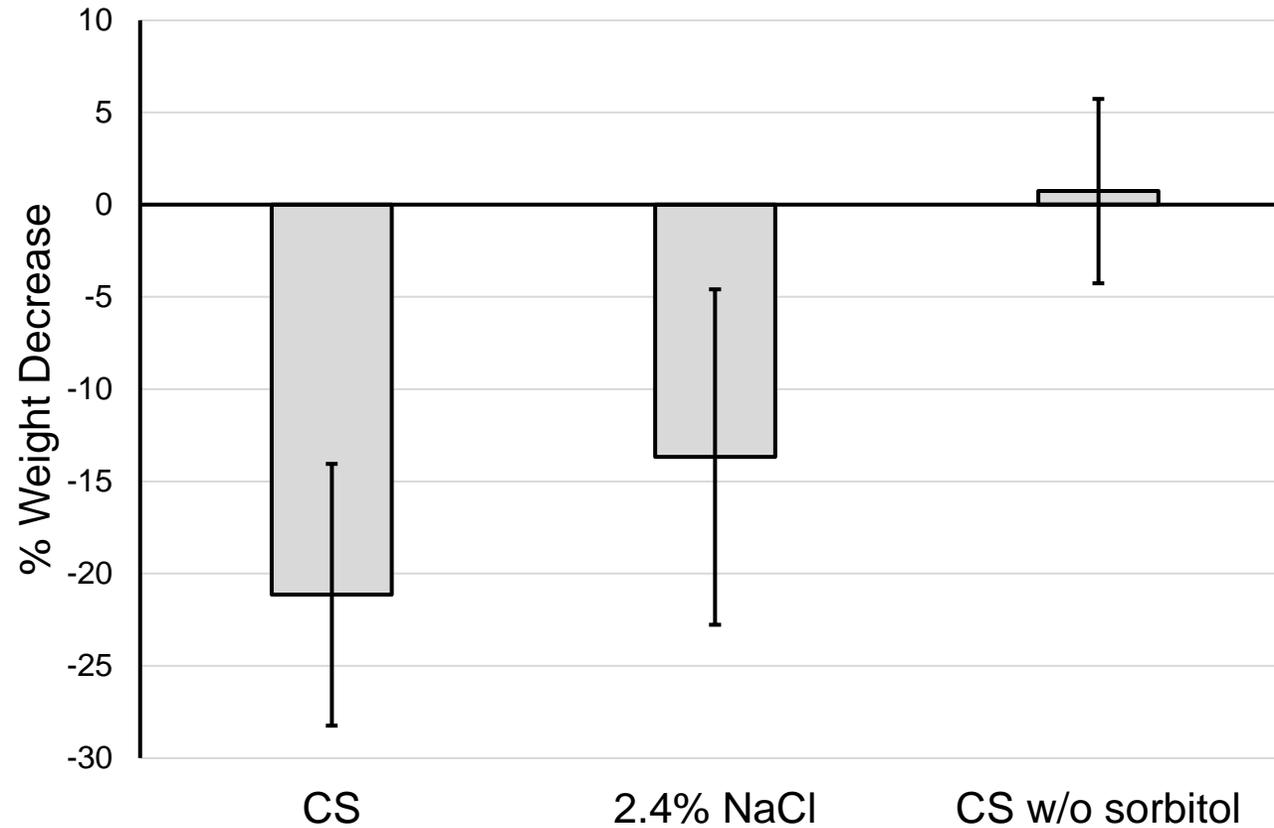


Figure 2: In vitro assay: Barrier function of CS nasal spray. Results of the percentage blocking activity of CS nasal spray relative to negative control (contains sorbitol and NaCl in same concentration as in CS but does not contain the barrier forming component carrageenan). Amounts of barrier-crossing beads were analyzed 180 minutes after application of beads. Cyan = % blocking activity for bead size of 0.3 μm ; blue = % blocking activity for bead size of 1.0 μm . Error bars represent standard deviation of replicates.

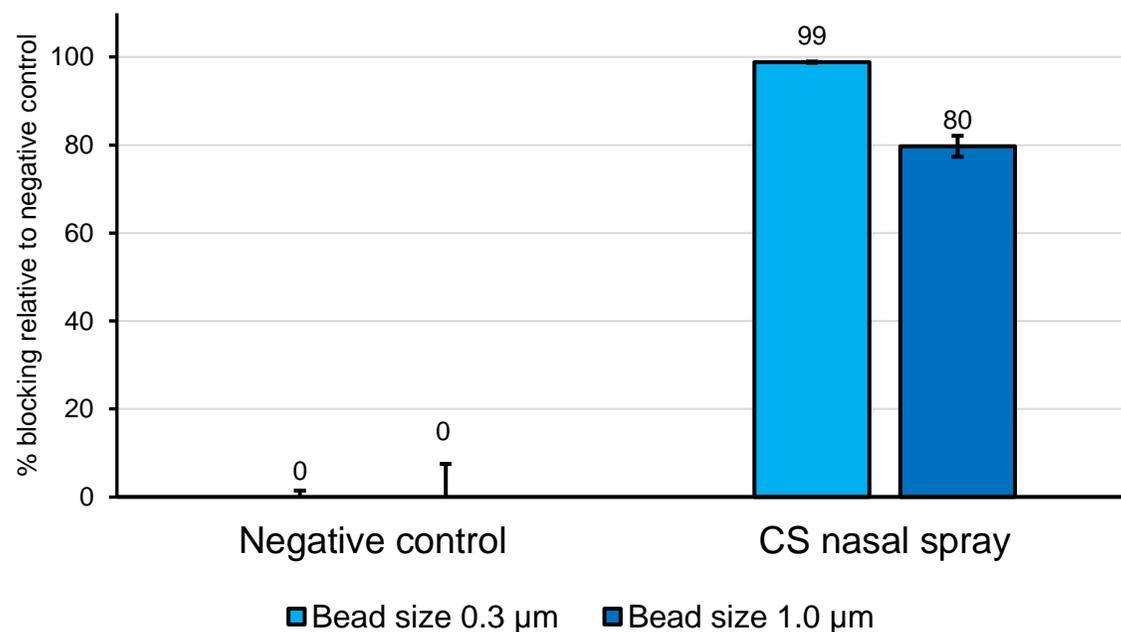


Figure 3: Clinical Study: Graphical Abstract
Panel A: Study Overview

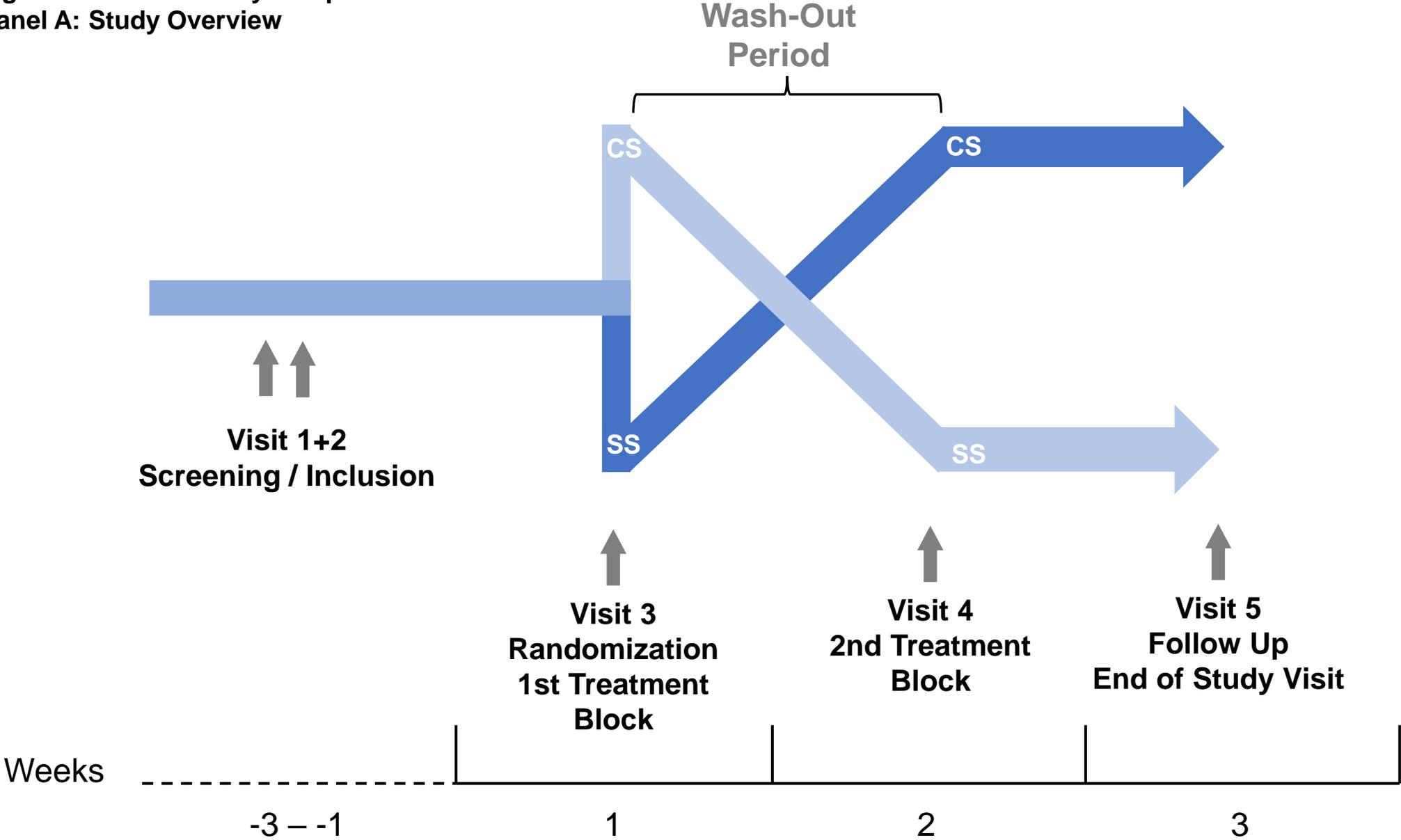


Figure 3: Clinical Study: Graphical Abstract
Panel B: Efficacy assessments carried out per treatment block.

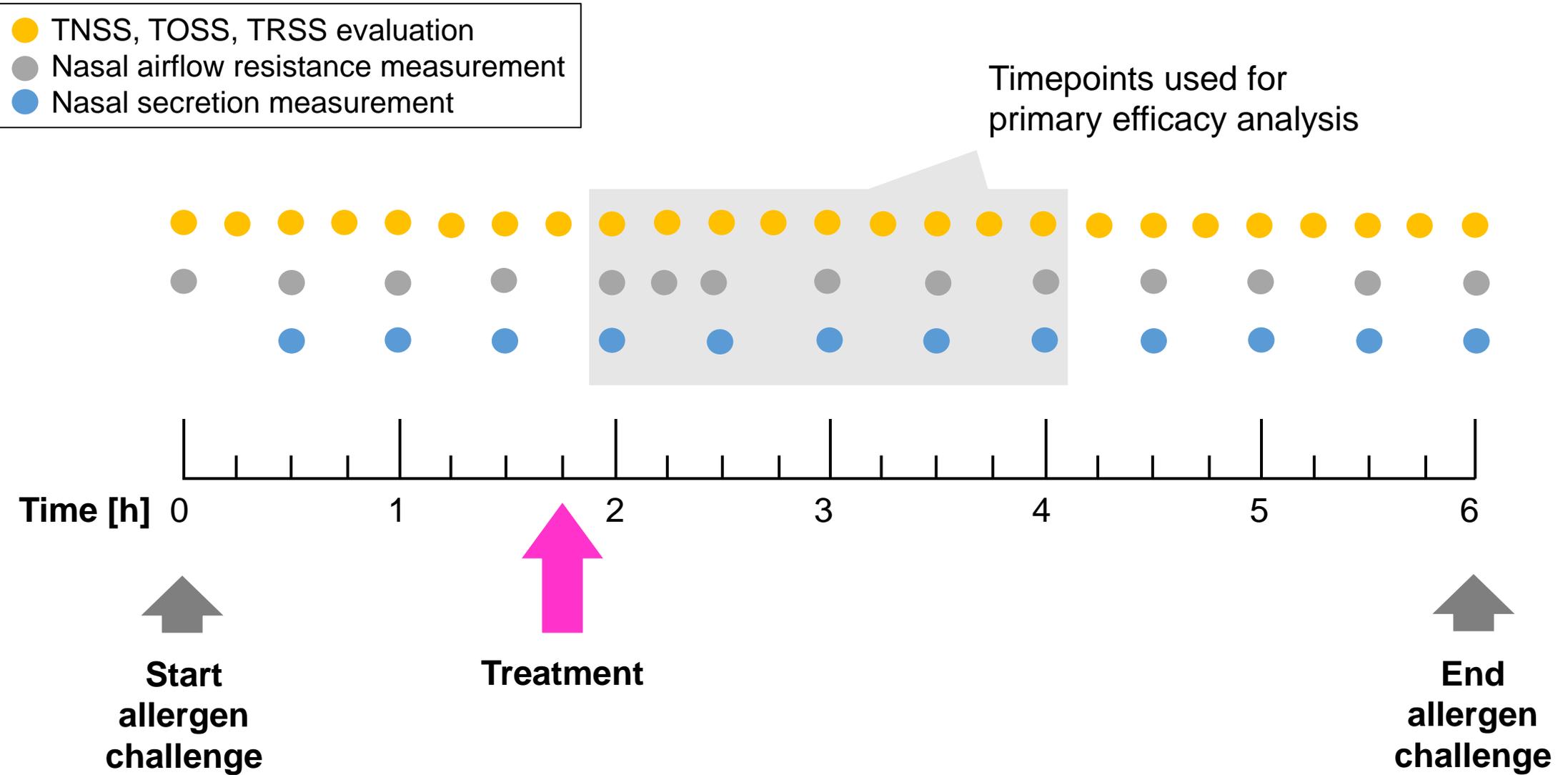


Figure 4: Clinical Study: CONSORT Flow Chart

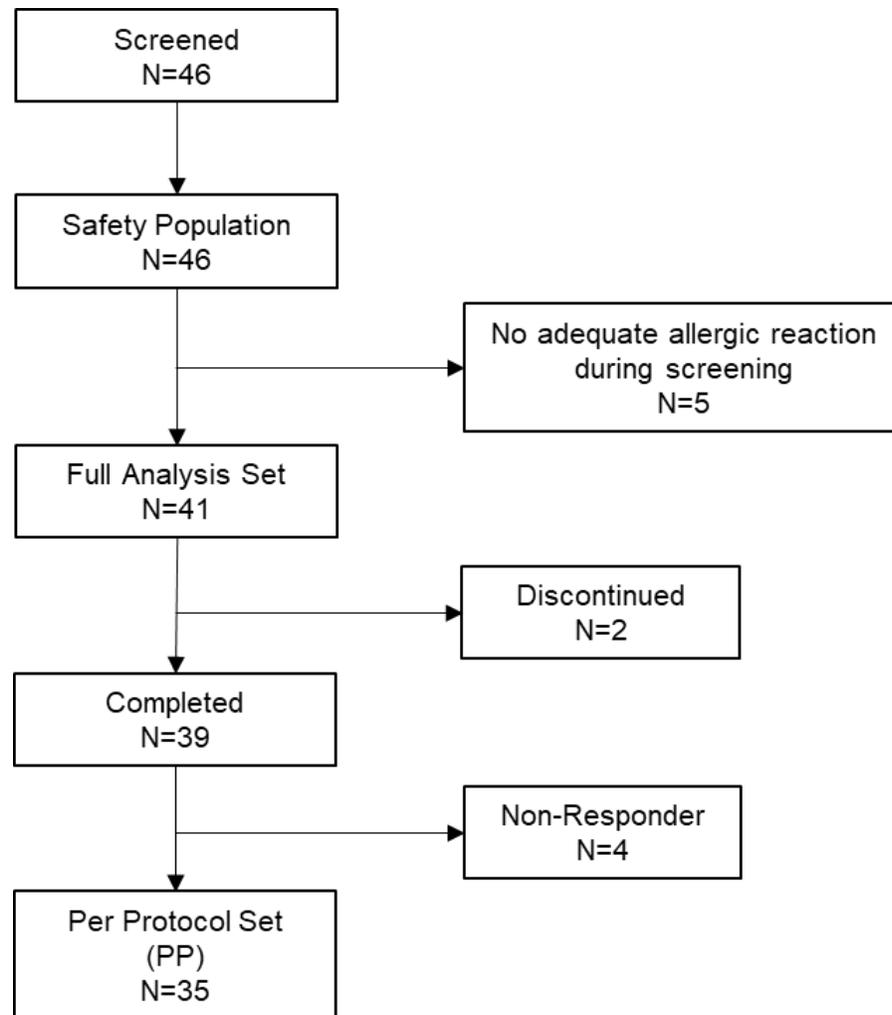
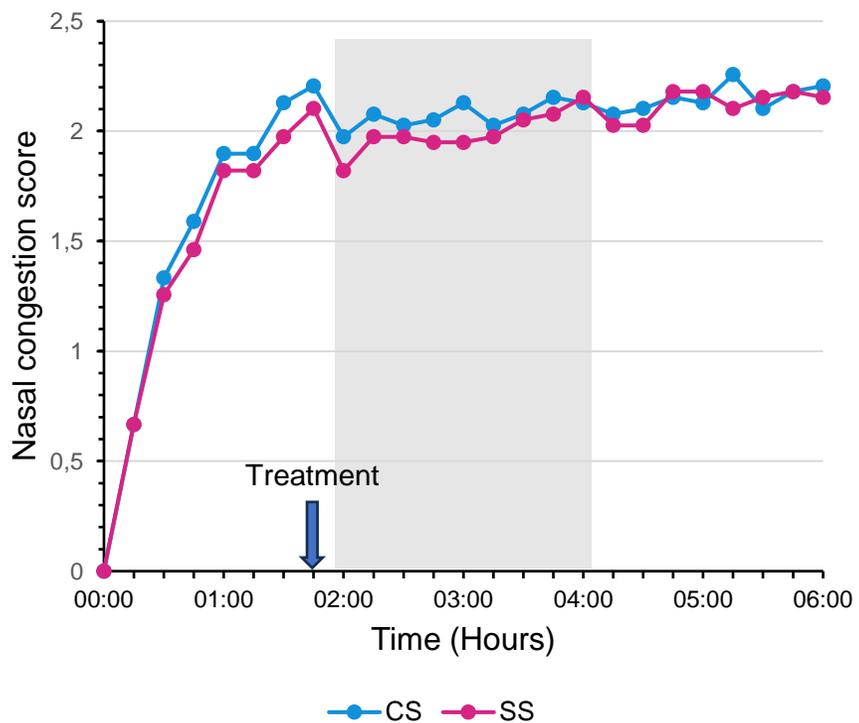


Figure 5: Clinical data: Nasal Congestion Symptom Score (NCSS) pre- and post-treatment during the grass pollen allergen exposure challenge for the FAS.

Panel A: Baseline corrected mean time course of nasal NCSS. The gray square highlights the timepoints used for the primary efficacy analysis.



Panel B: Primary efficacy analysis: Mean difference of treatments (Mean NCSS Δ [Pre-treatment - \emptyset (2-4h)]) and 95% CI for the FAS. The mean difference of CS – SS = 0.02, 95% CI [-0.19;0.24], $p > 0.05$ (paired t-test).

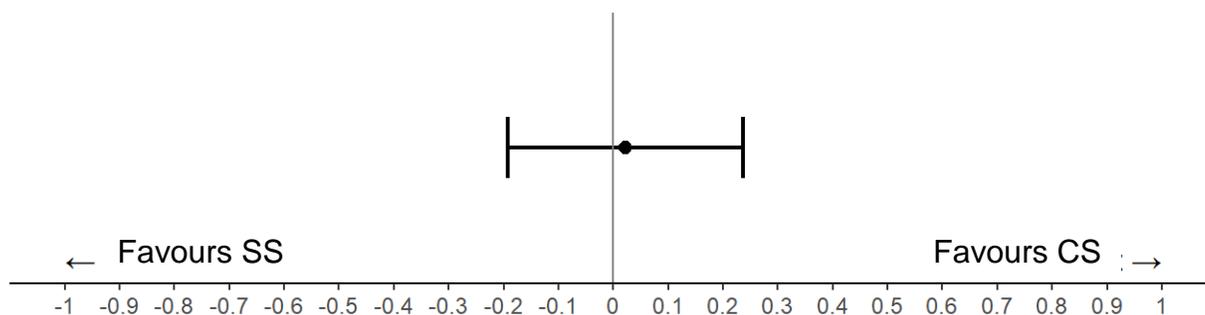


Figure 6: Clinical data: Anterior nasal airflow before and after treatment for the FAS. Mean airflow at timepoints 1h30min (before treatment) and 6h after start of allergen challenge. Error bars denote 95% CI. $P=0.039$ for comparison between treatments in difference from pre-treatment to timepoint 360 min.

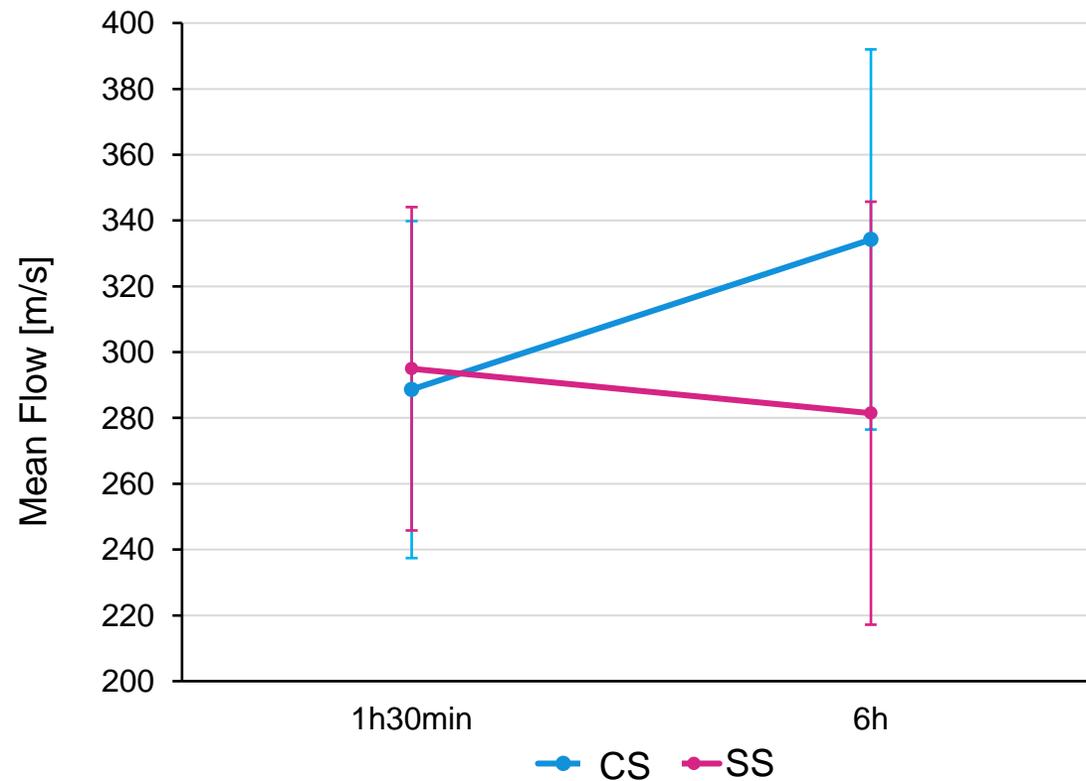
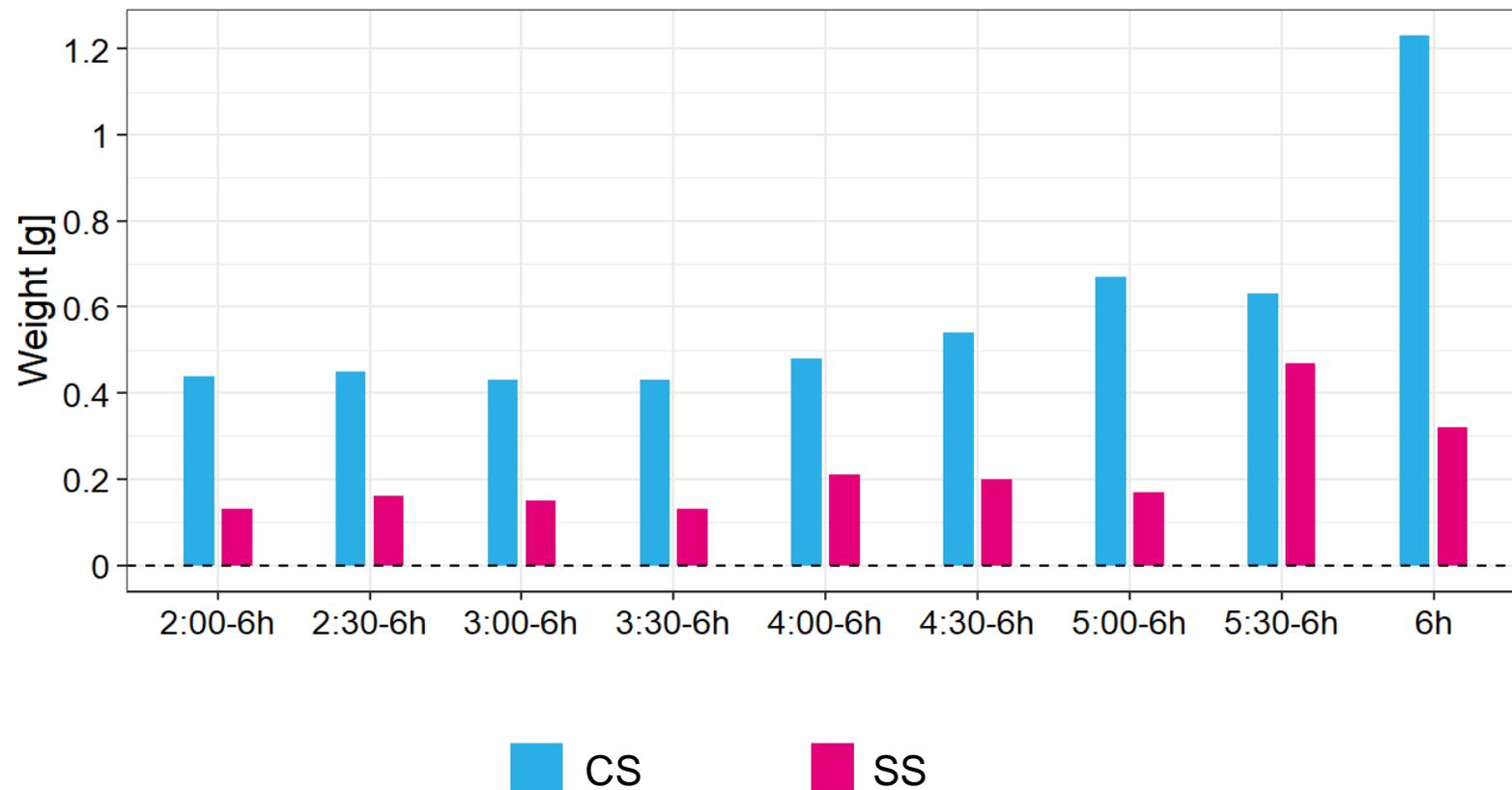


Figure 7: Clinical data: Median nasal secretion absolute differences to pre-treatment for the FAS. Differences in post-treatment nasal secretion compared to pre-treatment after CS treatment (cyan) and saline treatment (magenta). Positive values indicate lower nasal secretion post-treatment compared to pre-treatment.



Supplemental Figure S1: Clinical data: Median anterior nasal airflow absolute differences to pre-treatment for the FAS. Differences in post-treatment nasal airflow compared to pre-treatment in the CS group (cyan) and the SS (magenta). Positive values indicate higher nasal airflow post-treatment, negative value indicate lower nasal airflow post-treatment compared to pre-treatment.

