

Nicotinamide modulates gut microbial metabolic potential and accelerates recovery in mild-to-moderate COVID-19

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SUPPLEMENT

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1. Summary of the pilot trial COVit-1

The pilot trial COVit-1 was a double-blind, randomized, controlled, monocentric trial conducted at the Department of Internal Medicine I of the University Hospital Schleswig-Holstein (Kiel, Germany). The protocol (see section 5 of this Supplement) including all amendments was approved by the Ethics Committee of the Medical Faculty of Kiel University (file reference A107/20) and all patients gave informed consent prior to any study procedures (see below). COVit-1 and COVit-2 were registered with the WHO primary registry German Clinical Trials Register (DRKS00021214). The trial was conducted remotely (while patients were confined to domestic quarantine) using the same procedures as described for COVit-2, but without biosampling. The trial population of COVit-1 is slightly different from the population of COVit-2 due to the recruitment of participants from physician's practices in the referral network of the University Hospital Schleswig-Holstein. In contrast to recruiting via diagnostic laboratories as in COVit-2, this may have resulted in a more severely affected patient population.

In the COVit-1 trial, 56 outpatients mainly from the Kiel area with early symptomatic COVID-19 in domestic quarantine were recruited from 06 April 2020 to 28 January 2021. The trial was double blind and patients were randomized 1:1 to either receive orally 1,000 mg of nicotinamide (NicoPel®, Derma Enzinger, Ainring, Germany) or 245 mg silica (Salus Pharma, Bruckmühl, Germany). The study intervention was taken once daily with breakfast in the morning, for 4 weeks (28 days).

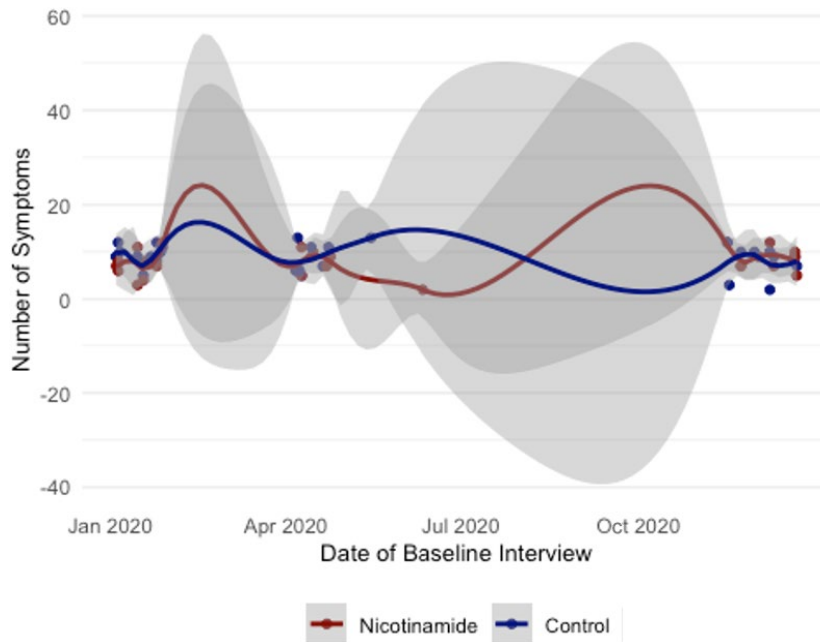
Inclusion criteria were a laboratory-confirmed SARS-CoV-2 infection, COVID-19 symptoms in the respiratory or gastrointestinal tract and an age of ≥ 18 years. There were no formal exclusion criteria. Supplementary Table 1 shows the baseline characteristics of the COVit-1 trial population (intention-to-treat, ITT) in the same format as used for COVit-2 (Table 1). Virus variants were not systematically determined, but the timeframe of recruitment of COVit-1 comprised mostly the wildtype strain of the pandemic, as the Alpha variant (B.1.1.7) was first detected in the recruitment area in the second week of January, 2021.

Supplementary Table 1: Demographic and clinical characteristics of all patients at baseline (intention-to-treat population) in the pilot trial COVit-1.

| Characteristics | Nicotinamide (n=28) | Control (n=28) | Total (n=56) |
|--|------------------------|-------------------|-----------------|
| Median age (range) at randomization – yr | 40 (18–69) | 46 (20–58) | 42 (18–69) |
| Sex – no. of patients (%) | | | |
| Male | 11 (39.3) | 10 (35.7) | 21 (37.5) |
| Female | 17 (60.7) | 18 (64.3) | 35 (62.5) |
| Race or ethnic group – no. of patients (%) | | | |
| White | 28 (100) | 25 (89.3) | 53 (94.6) |
| Other | 0 (0) | 3 (10.7) | 3 (5.4) |
| Not reported | 0 (0) | 0 (0) | 0 (0) |
| Body mass index (BMI) (mean \pm SD) | 26.1 (4.5) | 26.3 (6.6) | 26.2 (5.6) |
| Risk factors for severe COVID-19 – no. (%) | | | |
| Age ≥ 60 yr | 4 (14.3) | 0 (0) | 4 (7.1) |
| BMI ≥ 30 and/or type 2 diabetes | 4 (14.3) | 5 (17.9) | 9 (16.1) |
| Cardiovascular diseases, high blood pressure or stroke | 7 (25.0) | 5 (17.9) | 12 (21.4) |
| Asthma, COPD or other chronic lung diseases | 6 (21.4) | 0 (0) | 6 (10.7) |
| Current or former smokers | 18 (64.3) | 23 (82.1) | 41 (73.2) |
| Other risk factors | 10 (35.7) | 11 (39.3) | 21 (37.5) |
| At least one risk factor | 26 (92.9) | 27 (96.4) | 53 (94.6) |

As shown in Supplementary Table 1, 26/28 (93%) in the nicotinamide and 27/28 (96%) of the patients in the control group of the COVit-1 trial had at least one risk factor for severe COVID-19, although this was not an inclusion criterion. In COVit-2, patients with at least one risk factor for severe COVID-19 were less frequent. The RFITT population of the COVit-2 trial comprised 248/430 (58%) of such patients in the nicotinamide group and 252/437 (also 58%) in the placebo group (cf. Table 1, Supplementary Table 3 and Extended Data Fig. 2). This difference in patient attraction was probably due to the different recruitment strategies: physician recruitment in COVit-1, enlisting participants who had shown up at local therapeutic facilities including the emergency rooms but then remained outpatients, is contrasted by COVit-2 with recruitment of patients who freshly received a positive test result for SARS-CoV-2 infection from a diagnostic laboratory (see section 3.1 of this Supplement).

The patients in the COVit-1 ITT population (n=56) showed a different symptomatology compared to the patients in the COVit-2 ITT (n=867) with significantly more fever and pain at baseline (Supplementary Table 2). Interestingly, the number of symptoms per patient at baseline was only slightly higher in COVit-1 (Supplementary Fig. 1) in comparison with COVit-2 (Supplementary Fig. 9). The recruitment period of COVit-2 from 01 February 2021 until 17 January 2022 covered the end of the Alpha (B.1.1.7) wave, the entire Delta (B.1.617.2) wave and the beginning of the first Omicron (B.1.1.529) wave in Germany.



Supplementary Fig. 1: Number of symptoms reported at baseline in the intention-to-treat population of the pilot trial COVIT-1.

Each dot represents one patient (n=56). Lines were produced with locally weighted scatterplot (LOESS) smoothing in R. Grey shades are 95% CI bounds of LOESS smoothing.

Supplementary Table 2: Baseline symptoms of the intention-to-treat population of the pilot trial COVIT-1 compared to symptom frequencies in the COVIT-2 trial.

| Symptoms | Nicotinamide (n=28) no. (%) | Control (n=28) no. (%) | P* | COVIT-1 ITT (n=56) no. (%) | COVIT-2 ITT (n=867) no. (%) | P** |
|-------------------------|-----------------------------------|------------------------------|--------------|----------------------------------|-----------------------------------|-------------------|
| Fatigue | 23 (82.1) | 26 (92.9) | 0.422 | 49 (87.5) | 663 (76.5) | 0.0696 |
| Performance drop | 23 (82.1) | 23 (82.1) | 1.000 | 46 (82.1) | 617 (71.2) | 0.0913 |
| Fever*** | 14 (50.0) | 17 (60.7) | 0.420 | 31 (55.4) | 117 (13.5) | <0.0001 |
| Shortness of breath | 8 (28.6) | 18 (35.7) | 0.567 | 26 (46.4) | 268 (30.9) | 0.0182 |
| Cough | 20 (71.4) | 16 (57.1) | 0.265 | 36 (64.3) | 426 (49.1) | 0.0378 |
| Cough with sputum | 7 (25.0) | 9 (32.1) | 0.554 | 16 (28.6) | 312 (36.0) | 0.3138 |
| Head cold (rhinorrhea) | 22 (78.6) | 22 (78.6) | 1.000 | 44 (78.6) | 634 (73.1) | 0.4366 |
| Sore throat | 10 (35.7) | 21 (75.0) | 0.003 | 31 (55.4) | 334 (38.5) | 0.0160 |
| Muscle pain | 14 (50.0) | 11 (39.3) | 0.420 | 25 (44.6) | 250 (28.8) | 0.0156 |
| Joint pain | 10 (35.7) | 13 (46.4) | 0.415 | 23 (41.1) | 177 (20.4) | 0.0007 |
| Chest pain | 7 (25.0) | 11 (39.3) | 0.252 | 18 (32.1) | 156 (18.0) | 0.0130 |
| Headache | 22 (78.6) | 24 (85.7) | 0.729 | 46 (82.1) | 398 (45.9) | <0.0001 |
| Diarrhea | 8 (28.6) | 5 (17.9) | 0.342 | 13 (23.2) | 117 (13.5) | 0.0486 |
| Vomiting | 1 (3.6) | 5 (17.9) | 0.193 | 6 (10.7) | 5 (0.6) | <0.0001 |
| Loss of appetite | 12 (42.9) | 17 (60.7) | 0.181 | 29 (51.8) | 414 (47.8) | 0.5832 |
| Impaired sense of smell | 17 (60.7) | 14 (50.0) | 0.420 | 31 (55.4) | 425 (49.0) | 0.4087 |
| Impaired sense of taste | 18 (64.3) | 14 (50.0) | 0.280 | 32 (57.1) | 395 (45.6) | 0.0984 |

* COVIT-1: nicotinamide vs. control; Chi-square test (one-sided) or Fisher exact test (two-sided), unadjusted.

** COVIT vs. COVIT-2, Fisher exact test (two-sided, unadjusted).

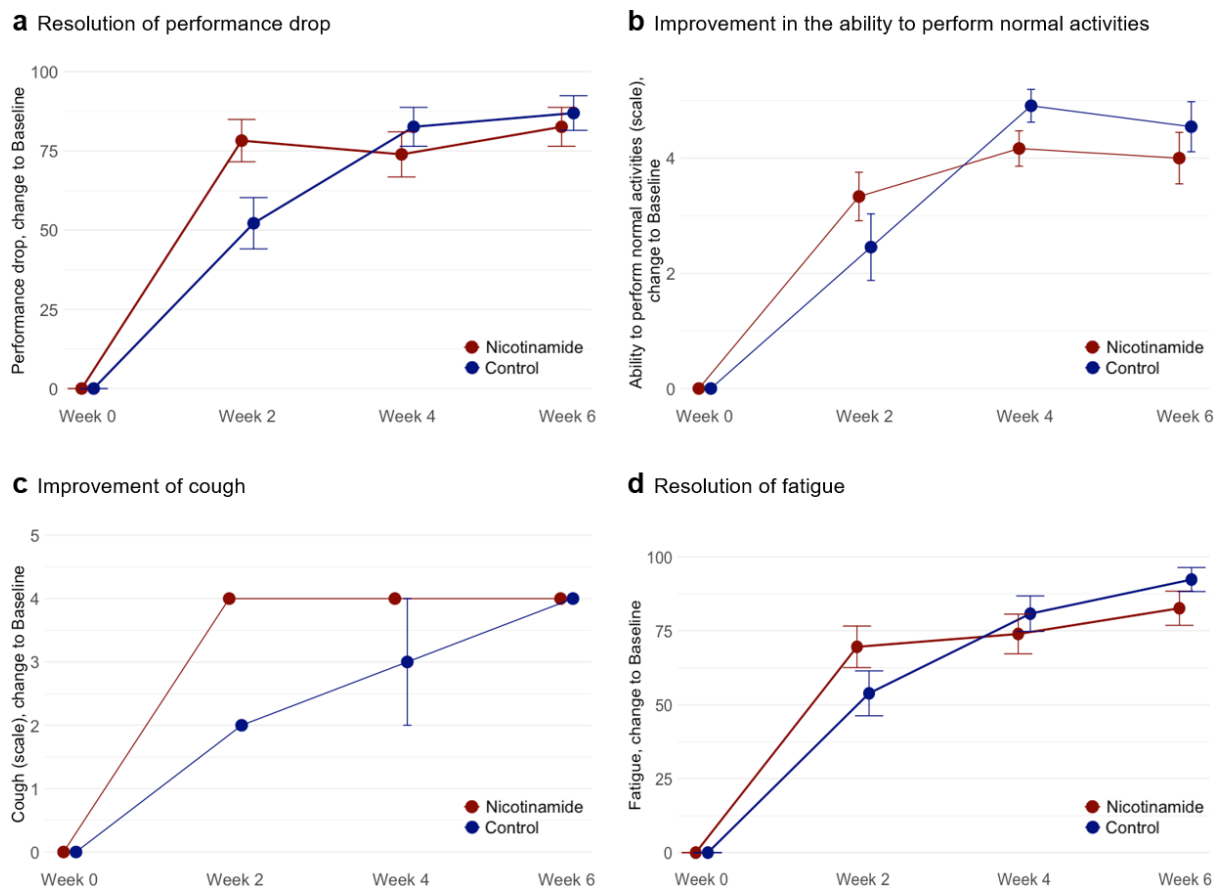
*** Missing temperature for 1 patient allocated to nicotinamide in COVIT-1.

Bold, fever and pain symptomatology.

COVIT-1 was started in the first weeks of the pandemic, when the number of patients requiring hospitalization and the numbers of infections were expected rise significantly. The planned primary endpoint of the COVIT-1 pilot trial was the frequency of hospital admission in order to receive at least 24 h of continuous oxygen therapy, and secondary endpoints included frequencies of machine ventilation, intensive care, death as well as time to resolution of symptoms. As blinded frequency analyses showed that the primary endpoint did not occur in a sufficiently high frequency, because the COVID-19 disease courses did not result in enough intensive care admissions and deaths, the trial was stopped. Symptom-related endpoints were observed in frequencies necessary for a statistical analysis, and complete resolution of symptoms after 2 weeks was defined as the principal goal for an exploratory, post-hoc

statistical analysis. Therefore, the COVit-1 trial was a pilot experiment. Protocol amendments were approved by the Ethics Committee and recruitment seamlessly continued with COVit-2 becoming a trial with a second patient population. Endpoints were adapted to the evolving real-world clinical situation, the number of recruitment sites was increased, and molecular analyses were included. The populations in COVit-1 and COVit-2 are completely separated and were not analyzed together.

Symptom frequencies and intensities at week 2 compared to baseline were more strongly reduced in the nicotinamide group than in the control group. The results for the post-hoc endpoints (cf. COVit-2 primary and key secondary endpoints) are shown for the COVit-1 ITT population (n=56) in Supplementary Fig. 2. Of note, complete resolution of symptoms after 2 weeks was substantially higher in the nicotinamide group (35.7% of patients) than in the control group (14.3%). The 35 female participants were main contributors to the difference between the nicotinamide and control interventions ($P=0.043$). Taken together, the results from COVit-1 warranted investigation of nicotinamide versus placebo in a larger and more definite trial.



Supplementary Fig. 2: Results of the primary and secondary endpoints of COVit-2 in a post-hoc analysis of the COVit-1 pilot trial intention-to-treat population.

Statistically significant differences (post-hoc) were observed for the resolution of performance drop (panel **a**, $P=0.034$) and the improvement in the ability to perform normal activities (panel **b**, $P=0.045$). In panel **c**, a standard error is only calculated for week 4 in the control group due to the low number of patients with severe cough at baseline (1 patient receiving nicotinamide and 2 patients receiving inert control, with scale improvements of -4 and -2 at week 4 compared to baseline, respectively). For the numbers of patients with the respective symptoms at baseline, see Supplementary Table 2. Graphs represent relative frequency \pm standard deviation (**a**, **d**) or mean \pm standard error (**b**, **c**).

2. Supplementary results for COVit-2

2.1. Demographic and clinical characteristics at baseline

Supplementary Table 3: Demographic and clinical characteristics of the patients at baseline (RFITT population).

| Characteristics | Nicotinamide (n=248) | Placebo (n=252) | Total (n=500) |
|--|-------------------------|--------------------|------------------|
| Median age (range) at randomization – yr | 38 (18–75) | 39 (18–70) | 39 (18–75) |
| Sex – no. of patients (%) | | | |
| Male | 101 (40.7) | 108 (42.9) | 209 (41.8) |
| Female | 147 (59.3) | 144 (57.1) | 291 (58.2) |
| Race or ethnic group – no. of patients (%) | | | |
| White | 238 (96.0) | 243 (96.4) | 481 (96.2) |
| Other | 10 (4.0) | 9 (3.6) | 19 (3.8) |
| Not reported | 0 (0) | 0 (0.0) | 0 (0) |
| Body mass index (BMI) (mean ± SD) | 26.3 ± 5.5 | 27.2 ± 5.4 | 26.8 ± 5.5 |
| Risk factors for severe COVID-19 – no. (%) | | | |
| Age ≥ 60 yr | 22 (8.9) | 20 (7.9) | 42 (8.4) |
| BMI ≥ 30 and/or type 2 diabetes | 58 (23.4) | 73 (29.0) | 131 (26.2) |
| Cardiovascular diseases, high blood pressure or stroke | 63 (25.4) | 60 (23.8) | 123 (24.6) |
| Asthma, chronic obstructive pulmonary disease or other chronic lung diseases | 41 (16.5) | 52 (20.6) | 93 (18.6) |
| Current or former smokers | 165 (66.5) | 171 (67.9) | 336 (67.2) |
| Other risk factors | 21 (8.5) | 15 (6.0) | 36 (7.2) |

The primary analysis population RFITT consists of patients with at least one risk factor for severe COVID-19 (see section 3.4 of this Supplement).

2.2. Supplementary clinical data (tables)

Supplementary Table 4: Descriptive statistics of SF-36 scales in the RFITT population.

| Time | Scale | Nicotinamide (n=248) | | Placebo (n=252) | | Total (n=500) | |
|--------|----------------------------|----------------------|--------------------|-----------------|--------------------|---------------|--------------------|
| | | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| Week 0 | Physical functioning | 53.50 | 27.50 | 51.19 | 26.80 | 52.09 | 27.21 |
| | Role functioning/physical | 48.68 | 45.19 | 53.04 | 44.43 | 50.73 | 44.83 |
| | Role functioning/emotional | 69.87 | 40.93 | 70.95 | 40.26 | 69.69 | 40.83 |
| | Energy/fatigue | 50.72 | 21.20 | 52.17 | 21.66 | 50.97 | 21.52 |
| | Emotional well-being | 67.42 | 18.09 | 69.20 | 17.78 | 67.77 | 18.10 |
| | Social functioning | 62.20 | 30.23 | 63.32 | 29.81 | 62.64 | 29.99 |
| | Pain | 64.46 | 29.13 | 64.51 | 30.33 | 64.02 | 29.78 |
| | General health | 60.02 | 18.31 | 59.82 | 18.38 | 59.72 | 18.37 |
| | Health change | 23.80 | 23.02 | 23.36 | 25.24 | 23.42 | 24.03 |
| Week 2 | Physical functioning | 75.25 | 25.03 | 74.13 | 26.17 | 74.11 | 25.85 |
| | Role functioning/physical | 34.25 | 40.99 | 28.97 | 38.78 | 31.55 | 40.04 |
| | Role functioning/emotional | 57.21 | 44.82 | 56.23 | 44.99 | 56.92 | 44.93 |
| | Energy/fatigue | 44.48 | 22.43 | 44.02 | 20.67 | 43.97 | 21.57 |
| | Emotional well-being | 65.62 | 18.73 | 66.29 | 18.69 | 65.55 | 18.84 |
| | Social functioning | 51.20 | 29.36 | 50.18 | 28.06 | 50.48 | 28.71 |
| | Pain | 58.49 | 27.88 | 55.68 | 28.18 | 57.04 | 28.09 |
| | General health | 63.91 | 18.65 | 62.53 | 18.99 | 62.74 | 19.02 |
| | Health change | 33.17 | 22.10 | 33.06 | 23.16 | 32.90 | 22.62 |
| Week 4 | Physical functioning | 86.19 | 19.11 | 85.28 | 20.32 | 84.96 | 20.40 |
| | Role functioning/physical | 51.84 | 43.17 | 47.20 | 43.78 | 48.89 | 43.62 |
| | Role functioning/emotional | 68.11 | 42.13 | 66.04 | 42.97 | 66.52 | 42.87 |
| | Energy/fatigue | 53.25 | 22.66 | 52.83 | 21.19 | 52.77 | 21.90 |
| | Emotional well-being | 69.75 | 18.82 | 70.55 | 19.05 | 69.96 | 18.87 |
| | Social functioning | 64.18 | 27.56 | 64.66 | 25.70 | 64.06 | 26.89 |
| | Pain | 72.93 | 25.15 | 69.47 | 26.36 | 70.94 | 25.77 |
| | General health | 68.11 | 19.11 | 65.33 | 18.69 | 66.53 | 18.96 |
| | Health change | 39.78 | 20.58 | 40.65 | 21.81 | 40.08 | 21.10 |

| Time | Scale | Nicotinamide (n=248) | | Placebo (n=252) | | Total (n=500) | |
|--------|----------------------------|----------------------|--------------------|-----------------|--------------------|---------------|--------------------|
| | | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| Week 6 | Physical functioning | 90.67 | 15.24 | 90.74 | 17.32 | 90.50 | 16.28 |
| | Role functioning/physical | 69.07 | 40.06 | 67.52 | 40.20 | 67.77 | 40.19 |
| | Role functioning/emotional | 77.88 | 35.47 | 75.55 | 38.21 | 76.16 | 37.26 |
| | Energy/fatigue | 62.12 | 21.55 | 61.64 | 20.63 | 61.56 | 21.10 |
| | Emotional well-being | 75.42 | 17.54 | 75.36 | 17.89 | 75.21 | 17.68 |
| | Social functioning | 79.03 | 22.28 | 76.29 | 23.58 | 77.27 | 23.11 |
| | Pain | 86.30 | 20.21 | 81.23 | 23.64 | 83.31 | 22.36 |
| | General health | 70.36 | 19.57 | 69.42 | 18.86 | 69.86 | 19.08 |
| | Health change | 45.55 | 20.15 | 46.73 | 21.42 | 46.13 | 20.67 |

The higher the scores, the better the quality of life. The range for each of the eight SF-36 multi-item scales (except for health change) is 0 – 100. For details on scoring and reference ranges, see https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form/scoring.html (last accessed on 11-Mar-2025).

Supplementary Table 5: Descriptive statistics of FACIT-F scales in the RFITT population.

| Time | Scale | Nicotinamide (n=248) | | Placebo (n=252) | | Total (n=500) | |
|--------|--------------------------|----------------------|--------------------|-----------------|--------------------|---------------|--------------------|
| | | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| Week 0 | Physical well-being | 15.96 | 5.70 | 14.86 | 5.86 | 15.29 | 5.93 |
| | Social/family well-being | 22.35 | 4.47 | 21.97 | 4.56 | 22.30 | 4.51 |
| | Emotional well-being | 18.16 | 3.86 | 18.29 | 4.30 | 18.13 | 4.15 |
| | Functional well-being | 13.34 | 5.69 | 12.97 | 5.85 | 13.03 | 5.80 |
| | Fatigue | 23.33 | 12.30 | 22.59 | 11.98 | 22.99 | 12.21 |
| | Trial Outcome Index | 52.63 | 20.92 | 50.42 | 20.54 | 51.37 | 21.08 |
| | FACT G Score | 69.82 | 13.70 | 68.09 | 14.49 | 68.67 | 14.21 |
| | FACIT-F General Score | 93.15 | 24.01 | 90.68 | 24.08 | 91.57 | 24.35 |
| Week 2 | Physical well-being | 22.69 | 5.35 | 22.03 | 5.29 | 22.29 | 5.40 |
| | Social/family well-being | 22.60 | 4.18 | 21.74 | 4.27 | 22.11 | 4.46 |
| | Emotional well-being | 20.32 | 3.46 | 20.03 | 3.62 | 19.99 | 3.62 |
| | Functional well-being | 17.20 | 5.97 | 17.28 | 5.91 | 17.05 | 6.17 |
| | Fatigue | 35.35 | 13.24 | 34.68 | 12.41 | 34.61 | 12.82 |
| | Trial Outcome Index | 75.24 | 23.20 | 73.98 | 21.72 | 74.43 | 22.71 |
| | FACT G Score | 82.80 | 14.54 | 81.07 | 15.29 | 81.63 | 15.39 |
| | FACIT-F General Score | 118.15 | 26.65 | 115.75 | 26.24 | 116.50 | 26.91 |
| Week 4 | Physical well-being | 25.26 | 3.68 | 24.79 | 4.27 | 24.88 | 4.24 |
| | Social/family well-being | 22.83 | 4.80 | 22.39 | 4.52 | 22.56 | 4.78 |
| | Emotional well-being | 21.27 | 3.09 | 21.18 | 3.18 | 21.08 | 3.27 |
| | Functional well-being | 20.33 | 5.64 | 20.11 | 5.42 | 19.97 | 5.84 |
| | Fatigue | 41.47 | 10.05 | 41.32 | 9.96 | 41.08 | 10.42 |
| | Trial Outcome Index | 87.06 | 17.96 | 86.22 | 18.17 | 86.08 | 19.00 |
| | FACT G Score | 89.68 | 14.08 | 88.47 | 13.66 | 88.51 | 14.49 |
| | FACIT-F General Score | 131.16 | 22.87 | 129.79 | 22.32 | 129.69 | 23.56 |

| Time | Scale | Nicotinamide | | Placebo | | Total | |
|--------|--------------------------|--------------|--------------------|---------|--------------------|--------|--------------------|
| | | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| Week 6 | Physical well-being | 26.16 | 2.98 | 26.11 | 3.46 | 26.02 | 3.31 |
| | Social/family well-being | 23.35 | 4.62 | 22.77 | 4.52 | 23.03 | 4.67 |
| | Emotional well-being | 21.75 | 2.68 | 21.70 | 3.17 | 21.61 | 2.95 |
| | Functional well-being | 21.67 | 5.36 | 22.06 | 5.24 | 21.71 | 5.37 |
| | Fatigue | 44.30 | 9.00 | 44.23 | 9.68 | 44.10 | 9.18 |
| | Trial Outcome Index | 92.13 | 15.97 | 92.40 | 17.15 | 92.02 | 16.38 |
| | FACT G Score | 92.92 | 12.92 | 92.64 | 13.52 | 92.60 | 13.28 |
| | FACIT-F General Score | 137.22 | 20.75 | 136.87 | 22.04 | 136.94 | 21.09 |

The higher the scores, the better the quality of life. The ranges of the FACIT-F scales are as follows:

| Scale | Range |
|--------------------------|---------|
| Physical well-being | 0 – 28 |
| Social/family well-being | 0 – 28 |
| Emotional well-being | 0 – 24 |
| Functional well-being | 0 – 28 |
| Fatigue | 0 – 52 |
| Trial Outcome Index | 0 – 108 |
| FACT G Score | 0 – 108 |
| FACIT-F General Score | 0 – 160 |

For details on scoring and reference ranges, see <https://www.facit.org/measures/FACIT-F> (last accessed on 11-Mar-2025).

Supplementary Table 6: Effect of sex on key binary endpoints in the RFITT population.

| | Performance drop (primary endpoint) | | Fatigue (3 rd key secondary endpoint) | |
|-----------------|--|---------|---|---------|
| | Odds ratio | P value | Odds ratio | P value |
| Female vs. male | 1.30 | 0.392 | 0.98 | 0.957 |
| Treatment:Sex | 0.96 | 0.827 | 1.26 | 0.311 |

Male: n=209; female: n=291. Generalized logistic regression model, Wald test, two-sided, unadjusted.

Supplementary Table 7: Effect of sex on key ordinal endpoints in the RFITT population.

| | Ability to perform normal activities (1 st key secondary endpoint) | | Cough (2 nd key secondary endpoint) | |
|-----------------|--|---------|---|---------|
| | β | P value | β | P value |
| Female vs. male | 0.11 | 0.338 | -0.04 | 0.596 |
| Treatment:Sex | -0.02 | 0.750 | 0.03 | 0.512 |

Male: n=209; female: n=291. Mixed model for repeated measures, Wald test, two-sided, unadjusted.

When extending existing models for primary and key secondary endpoints by sex and treatment-sex interaction terms, no significant effects can be observed. Supplementary Tables 6 and 7 show odds ratio or regression coefficient (β), respectively, and corresponding P values of sex-specific dependent factors.

Supplementary Table 8: Effects of nicotinamide on COVID-19 symptoms (change to baseline) in the entire RFITT population (binary symptoms).

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Woolfe test | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|--------------------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|------|-------------|----|-------|------------------------------|----|-------|------------------------------|--------|--------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | X^2 | df | P | χ2MH | df | P | Week 2 | Week 4 | Week 6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Performance drop | Week 2 | 191 | 188 | 110 | 57.59% | 81 | 42.41% | 80 | 42.55% | 108 | 57.45% | 15.04% | 5.08% | 24.99% | 1.33 | 1.03 | 1.70 | 4.09 | 2 | 0.130 | 4.69 | 2 | 0.030 | 0.004 | 0.914 | 0.548 |
| | Week 4 | 191 | 188 | 126 | 65.97% | 65 | 34.03% | 123 | 65.43% | 65 | 34.57% | 0.54% | -9.02% | 10.10% | | | | | | | | | | | | |
| | Week 6 | 191 | 188 | 148 | 77.49% | 43 | 22.51% | 140 | 74.47% | 48 | 25.53% | 3.02% | -5.58% | 11.62% | | | | | | | | | | | | |
| Fatigue | Week 2 | 199 | 198 | 105 | 52.76% | 94 | 47.24% | 96 | 48.48% | 102 | 51.52% | 4.28% | -5.55% | 14.11% | 1.05 | 0.82 | 1.35 | 1.24 | 2 | 0.538 | 0.10 | 2 | 0.750 | 0.423 | 0.737 | 0.532 |
| | Week 4 | 199 | 198 | 146 | 73.37% | 53 | 26.63% | 142 | 71.72% | 56 | 28.28% | 1.65% | -7.13% | 10.43% | | | | | | | | | | | | |
| | Week 6 | 199 | 198 | 156 | 78.39% | 43 | 21.61% | 161 | 81.31% | 37 | 18.69% | -2.92% | -10.81% | 4.97% | | | | | | | | | | | | |
| Fever | Week 2 | 25 | 37 | 25 | 100.00% | 0 | 0.00% | 37 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| | Week 4 | 25 | 37 | 25 | 100.00% | 0 | 0.00% | 37 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| | Week 6 | 25 | 37 | 25 | 100.00% | 0 | 0.00% | 37 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| Chills | Week 2 | 24 | 29 | 24 | 100.00% | 0 | 0.00% | 29 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| | Week 4 | 24 | 29 | 24 | 100.00% | 0 | 0.00% | 29 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| | Week 6 | 24 | 29 | 24 | 100.00% | 0 | 0.00% | 29 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| Shortness of breath | Week 2 | 92 | 90 | 56 | 60.87% | 36 | 39.13% | 37 | 41.11% | 53 | 58.89% | 19.76% | 5.52% | 34.00% | 1.20 | 0.84 | 1.70 | 6.73 | 2 | 0.035 | 0.85 | 2 | 0.358 | 0.012 | 0.759 | 0.506 |
| | Week 4 | 92 | 90 | 58 | 63.04% | 34 | 36.96% | 59 | 65.56% | 31 | 34.44% | -2.51% | -16.43% | 11.40% | | | | | | | | | | | | |
| | Week 6 | 92 | 90 | 65 | 70.65% | 27 | 29.35% | 68 | 75.56% | 22 | 24.44% | -4.90% | -17.76% | 7.96% | | | | | | | | | | | | |
| Whistling / Wheezing breathing | Week 2 | 28 | 34 | 25 | 89.29% | 3 | 10.71% | 32 | 94.12% | 2 | 5.88% | -4.83% | -18.75% | 9.09% | 1.10 | 0.24 | 5.16 | 1.08 | 2 | 0.582 | 0.02 | 2 | 0.900 | 0.650 | >0.999 | >0.999 |
| | Week 4 | 28 | 34 | 28 | 100.00% | 0 | 0.00% | 33 | 97.06% | 1 | 2.94% | 2.94% | -2.74% | 8.62% | | | | | | | | | | | | |
| | Week 6 | 28 | 34 | 28 | 100.00% | 0 | 0.00% | 33 | 97.06% | 1 | 2.94% | 2.94% | -2.74% | 8.62% | | | | | | | | | | | | |
| Cough | Week 2 | 116 | 119 | 84 | 72.41% | 32 | 27.59% | 80 | 67.23% | 39 | 32.77% | 5.19% | -6.53% | 16.90% | 1.01 | 0.68 | 1.50 | 2.73 | 2 | 0.255 | 0.00 | 2 | 0.966 | 0.398 | 0.212 | 0.797 |
| | Week 4 | 116 | 119 | 94 | 81.03% | 22 | 18.97% | 104 | 87.39% | 15 | 12.61% | -6.36% | -15.66% | 2.94% | | | | | | | | | | | | |
| | Week 6 | 116 | 119 | 109 | 93.97% | 7 | 6.03% | 110 | 92.44% | 9 | 7.56% | 1.53% | -4.90% | 7.96% | | | | | | | | | | | | |
| Cough with sputum production | Week 2 | 93 | 95 | 72 | 77.42% | 21 | 22.58% | 74 | 77.89% | 21 | 22.11% | -0.48% | -12.39% | 11.43% | 0.71 | 0.42 | 1.20 | 2.29 | 2 | 0.318 | 1.33 | 2 | 0.250 | >0.999 | 0.061 | 0.719 |
| | Week 4 | 93 | 95 | 79 | 84.95% | 14 | 15.05% | 89 | 93.68% | 6 | 6.32% | -8.74% | -17.50% | 0.02% | | | | | | | | | | | | |
| | Week 6 | 93 | 95 | 89 | 95.70% | 4 | 4.30% | 92 | 96.84% | 3 | 3.16% | -1.14% | -6.56% | 4.28% | | | | | | | | | | | | |

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Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Woolfe test | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|---------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|------|-------------|----|--------|------------------------------|----|-------|------------------------------|--------|--------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | X^2 | df | P | χ2MH | df | P | Week 2 | Week 4 | Week 6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Rhinitis/rhinorrhea | Week 2 | 184 | 178 | 143 | 77.72% | 41 | 22.28% | 126 | 70.79% | 52 | 29.21% | 6.93% | -2.06% | 15.92% | 1.54 | 1.08 | 2.19 | 0.23 | 2 | 0.893 | 5.24 | 2 | 0.022 | 0.149 | 0.096 | 0.569 |
| | Week 4 | 184 | 178 | 164 | 89.13% | 20 | 10.87% | 147 | 82.58% | 31 | 17.42% | 6.55% | -0.61% | 13.71% | | | | | | | | | | | | |
| | Week 6 | 184 | 178 | 179 | 97.28% | 5 | 2.72% | 171 | 96.07% | 7 | 3.93% | 1.22% | -2.48% | 4.91% | | | | | | | | | | | | |
| Sore throat | Week 2 | 78 | 93 | 72 | 92.31% | 6 | 7.69% | 81 | 87.10% | 12 | 12.90% | 5.21% | -3.81% | 14.23% | 1.29 | 0.63 | 2.61 | 0.77 | 2 | 0.680 | 0.27 | 2 | 0.603 | 0.323 | >0.999 | >0.999 |
| | Week 4 | 78 | 93 | 71 | 91.03% | 7 | 8.97% | 85 | 91.40% | 8 | 8.60% | -0.37% | -8.90% | 8.15% | | | | | | | | | | | | |
| | Week 6 | 78 | 93 | 77 | 98.72% | 1 | 1.28% | 92 | 98.92% | 1 | 1.08% | -0.21% | -3.47% | 3.05% | | | | | | | | | | | | |
| Hoarseness | Week 2 | 76 | 92 | 67 | 88.16% | 9 | 11.84% | 86 | 93.48% | 6 | 6.52% | -5.32% | -14.16% | 3.52% | 0.89 | 0.38 | 2.08 | 3.86 | 2 | 0.145 | 0.00 | 2 | 0.967 | 0.281 | 0.129 | 0.452 |
| | Week 4 | 76 | 92 | 75 | 98.68% | 1 | 1.32% | 86 | 93.48% | 6 | 6.52% | 5.21% | -0.45% | 10.86% | | | | | | | | | | | | |
| | Week 6 | 76 | 92 | 75 | 98.68% | 1 | 1.32% | 92 | 100.00% | 0 | 0.00% | -1.32% | -3.88% | 1.25% | | | | | | | | | | | | |
| Pneumonia | Week 2 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| | Week 4 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| | Week 6 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | | | | |
| Muscle pain | Week 2 | 79 | 83 | 77 | 97.47% | 2 | 2.53% | 81 | 97.59% | 2 | 2.41% | -0.12% | -4.91% | 4.66% | 0.95 | 0.33 | 2.76 | 0.00 | 2 | >0.999 | 0.01 | 2 | 0.925 | >0.999 | >0.999 | >0.999 |
| | Week 4 | 79 | 83 | 75 | 94.94% | 4 | 5.06% | 79 | 95.18% | 4 | 4.82% | -0.24% | -6.92% | 6.43% | | | | | | | | | | | | |
| | Week 6 | 79 | 83 | 78 | 98.73% | 1 | 1.27% | 82 | 98.80% | 1 | 1.20% | -0.06% | -3.46% | 3.34% | | | | | | | | | | | | |
| Joint pain | Week 2 | 57 | 55 | 53 | 92.98% | 4 | 7.02% | 51 | 92.73% | 4 | 7.27% | 0.26% | -9.29% | 9.80% | 0.45 | 0.15 | 1.34 | 2.05 | 2 | 0.358 | 1.46 | 2 | 0.228 | >0.999 | 0.618 | 0.119 |
| | Week 4 | 57 | 55 | 54 | 94.74% | 3 | 5.26% | 54 | 98.18% | 1 | 1.82% | -3.44% | -10.23% | 3.34% | | | | | | | | | | | | |
| | Week 6 | 57 | 55 | 53 | 92.98% | 4 | 7.02% | 55 | 100.00% | 0 | 0.00% | -7.02% | -13.65% | -0.39% | | | | | | | | | | | | |
| Limb pain | Week 2 | 71 | 87 | 66 | 92.96% | 5 | 7.04% | 84 | 96.55% | 3 | 3.45% | -3.59% | -10.67% | 3.49% | 0.81 | 0.23 | 2.86 | 1.39 | 2 | 0.499 | 0.00 | 2 | 0.997 | 0.469 | >0.999 | >0.999 |
| | Week 4 | 71 | 87 | 71 | 100.00% | 0 | 0.00% | 86 | 98.85% | 1 | 1.15% | 1.15% | -1.09% | 3.39% | | | | | | | | | | | | |
| | Week 6 | 71 | 87 | 71 | 100.00% | 0 | 0.00% | 86 | 98.85% | 1 | 1.15% | 1.15% | -1.09% | 3.39% | | | | | | | | | | | | |
| Chest pain | Week 2 | 57 | 48 | 52 | 91.23% | 5 | 8.77% | 45 | 93.75% | 3 | 6.25% | -2.52% | -12.56% | 7.52% | 0.58 | 0.17 | 1.98 | 0.72 | 2 | 0.697 | 0.34 | 2 | 0.558 | 0.724 | 0.499 | >0.999 |
| | Week 4 | 57 | 48 | 55 | 96.49% | 2 | 3.51% | 48 | 100.00% | 0 | 0.00% | -3.51% | -8.29% | 1.27% | | | | | | | | | | | | |
| | Week 6 | 57 | 48 | 56 | 98.25% | 1 | 1.75% | 47 | 97.92% | 1 | 2.08% | 0.33% | -4.96% | 5.62% | | | | | | | | | | | | |
| Headache | Week 2 | 113 | 115 | 90 | 79.65% | 23 | 20.35% | 85 | 73.91% | 30 | 26.09% | 5.73% | -5.20% | 16.67% | 1.44 | 0.91 | 2.30 | 0.08 | 2 | 0.963 | 2.05 | 2 | 0.152 | 0.348 | 0.380 | 0.768 |
| | Week 4 | 113 | 115 | 104 | 92.04% | 9 | 7.96% | 101 | 87.83% | 14 | 12.17% | 4.21% | -3.58% | 12.00% | | | | | | | | | | | | |
| | Week 6 | 113 | 115 | 108 | 95.58% | 5 | 4.42% | 108 | 93.91% | 7 | 6.09% | 1.66% | -4.12% | 7.45% | | | | | | | | | | | | |

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Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Woolfe test | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|--------------------------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|-------|-------------|----|-------|------------------------------|----|-------|------------------------------|--------|--------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | X^2 | df | P | χ2MH | df | P | Week 2 | Week 4 | Week 6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Abdominal pain | Week 2 | 19 | 21 | 18 | 94.74% | 1 | 5.26% | 19 | 90.48% | 2 | 9.52% | 4.26% | -11.82% | 20.34% | 1.38 | 0.22 | 8.76 | 0.14 | 2 | 0.932 | 0.12 | 2 | 0.732 | >0.999 | >0.999 | >0.999 |
| | Week 4 | 19 | 21 | 19 | 100.00% | 0 | 0.00% | 21 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | |
| | Week 6 | 19 | 21 | 18 | 94.74% | 1 | 5.26% | 20 | 95.24% | 1 | 4.76% | -0.50% | -14.06% | 13.06% | | | | | | | | | | | | |
| Diarrhea | Week 2 | 28 | 51 | 26 | 92.86% | 2 | 7.14% | 45 | 88.24% | 6 | 11.76% | 4.62% | -8.39% | 17.63% | 1.40 | 0.42 | 4.65 | 0.14 | 2 | 0.934 | 0.07 | 2 | 0.790 | 0.705 | >0.999 | >0.999 |
| | Week 4 | 28 | 51 | 27 | 96.43% | 1 | 3.57% | 49 | 96.08% | 2 | 3.92% | 0.35% | -8.35% | 9.05% | | | | | | | | | | | | |
| | Week 6 | 28 | 51 | 27 | 96.43% | 1 | 3.57% | 49 | 96.08% | 2 | 3.92% | 0.35% | -8.35% | 9.05% | | | | | | | | | | | | |
| Nausea | Week 2 | 24 | 34 | 21 | 87.50% | 3 | 12.50% | 33 | 97.06% | 1 | 2.94% | -9.56% | -23.96% | 4.84% | 0.34 | 0.06 | 1.90 | 1.31 | 2 | 0.519 | 0.737 | 2 | 0.391 | 0.30 | >0.999 | 0.414 |
| | Week 4 | 24 | 34 | 24 | 100.00% | 0 | 0.00% | 33 | 97.06% | 1 | 2.94% | 2.94% | -2.74% | 8.62% | | | | | | | | | | | | |
| | Week 6 | 24 | 34 | 23 | 95.83% | 1 | 4.17% | 34 | 100.00% | 0 | 0.00% | -4.17% | -12.16% | 3.83% | | | | | | | | | | | | |
| Vomiting | Week 2 | 1 | 3 | 1 | 100.00% | 0 | 0.00% | 3 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 1 | 3 | 1 | 100.00% | 0 | 0.00% | 3 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | |
| | Week 6 | 1 | 3 | 1 | 100.00% | 0 | 0.00% | 3 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | |
| Loss of appetite / lower food intake | Week 2 | 125 | 126 | 118 | 94.40% | 7 | 5.60% | 116 | 92.06% | 10 | 7.94% | 2.34% | -3.87% | 8.54% | 1.48 | 0.72 | 3.05 | 0.00 | 2 | 0.999 | 0.78 | 2 | 0.377 | 0.617 | 0.749 | >0.999 |
| | Week 4 | 125 | 126 | 121 | 96.80% | 4 | 3.20% | 120 | 95.24% | 6 | 4.76% | 1.56% | -3.27% | 6.39% | | | | | | | | | | | | |
| | Week 6 | 125 | 126 | 123 | 98.40% | 2 | 1.60% | 123 | 97.62% | 3 | 2.38% | 0.78% | -2.67% | 4.23% | | | | | | | | | | | | |
| Other gastrointes-tinal symptoms | Week 2 | 11 | 7 | 9 | 81.82% | 2 | 18.18% | 6 | 85.71% | 1 | 14.29% | -3.90% | -38.41% | 30.62% | 2.58 | 0.38 | 17.50 | 1.17 | 2 | 0.558 | 0.28 | 2 | 0.597 | >0.999 | 0.389 | 0.389 |
| | Week 4 | 11 | 7 | 11 | 100.00% | 0 | 0.00% | 6 | 85.71% | 1 | 14.29% | 14.29% | -11.64% | 40.21% | | | | | | | | | | | | |
| | Week 6 | 11 | 7 | 11 | 100.00% | 0 | 0.00% | 6 | 85.71% | 1 | 14.29% | 14.29% | -11.64% | 40.21% | | | | | | | | | | | | |
| Impaired sense of smell | Week 2 | 114 | 137 | 61 | 53.51% | 53 | 46.49% | 67 | 48.91% | 70 | 51.09% | 4.60% | -7.80% | 17.01% | 1.19 | 0.88 | 1.63 | 1.37 | 2 | 0.505 | 1.08 | 2 | 0.299 | 0.526 | 0.892 | 0.179 |
| | Week 4 | 114 | 137 | 78 | 68.42% | 36 | 31.58% | 95 | 69.34% | 42 | 30.66% | -0.92% | -12.43% | 10.59% | | | | | | | | | | | | |
| | Week 6 | 114 | 137 | 92 | 80.70% | 22 | 19.30% | 100 | 72.99% | 37 | 27.01% | 7.71% | -2.67% | 18.09% | | | | | | | | | | | | |
| Impaired sense of taste | Week 2 | 103 | 137 | 56 | 54.37% | 47 | 45.63% | 76 | 55.47% | 61 | 44.53% | -1.11% | -13.83% | 11.61% | 1.11 | 0.81 | 1.54 | 0.95 | 2 | 0.623 | 0.33 | 2 | 0.567 | 0.896 | 0.776 | 0.281 |
| | Week 4 | 103 | 137 | 74 | 71.84% | 29 | 28.16% | 96 | 70.07% | 41 | 29.93% | 1.77% | -9.81% | 13.36% | | | | | | | | | | | | |
| | Week 6 | 103 | 137 | 83 | 80.58% | 20 | 19.42% | 102 | 74.45% | 35 | 25.55% | 6.13% | -4.44% | 16.70% | | | | | | | | | | | | |
| Confusion | Week 2 | 15 | 16 | 14 | 93.33% | 1 | 6.67% | 15 | 93.75% | 1 | 6.25% | -0.42% | -17.74% | 16.90% | 1.93 | 0.17 | 22.63 | 0.35 | 2 | 0.840 | 0.28 | 2 | 0.598 | >0.999 | >0.999 | >0.999 |
| | Week 4 | 15 | 16 | 15 | 100.00% | 0 | 0.00% | 15 | 93.75% | 1 | 6.25% | 6.25% | -5.61% | 18.11% | | | | | | | | | | | | |
| | Week 6 | 15 | 16 | 15 | 100.00% | 0 | 0.00% | 16 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | |

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Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Woolfe test | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|----------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|------|-------------|----|-------|------------------------------|----|--------|------------------------------|--------|--------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | X^2 | df | P | χ2MH | df | P | Week 2 | Week 4 | Week 6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Dizziness | Week 2 | 50 | 49 | 47 | 94.00% | 3 | 6.00% | 40 | 81.63% | 9 | 18.37% | 12.37% | -0.32% | 25.05% | 1.52 | 0.63 | 3.67 | 3.21 | 2 | 0.201 | 0.51 | 2 | 0.474 | 0.071 | 1.000 | 0.678 |
| | Week 4 | 50 | 49 | 48 | 96.00% | 2 | 4.00% | 47 | 95.92% | 2 | 4.08% | 0.08% | -7.68% | 7.84% | | | | | | | | | | | | |
| | Week 6 | 50 | 49 | 46 | 92.00% | 4 | 8.00% | 47 | 95.92% | 2 | 4.08% | -3.92% | -13.26% | 5.42% | | | | | | | | | | | | |
| Conjunctivitis | Week 2 | 3 | 9 | 2 | 66.67% | 1 | 33.33% | 9 | 100.00% | 0 | 0.00% | -33.33% | -86.68% | 20.01% | 0.33 | 0.02 | 5.33 | 1.12 | 2 | 0.571 | 0.00 | 2 | >0.999 | 0.250 | >0.999 | >0.999 |
| | Week 4 | 3 | 9 | 3 | 100.00% | 0 | 0.00% | 8 | 88.89% | 1 | 11.11% | 11.11% | -9.42% | 31.64% | | | | | | | | | | | | |
| | Week 6 | 3 | 9 | 3 | 100.00% | 0 | 0.00% | 9 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | |
| Skin rash | Week 2 | 17 | 11 | 15 | 88.24% | 2 | 11.76% | 11 | 100.00% | 0 | 0.00% | -11.76% | -27.08% | 3.55% | n/a | | | | | | | | | | | |
| | Week 4 | 17 | 11 | 16 | 94.12% | 1 | 5.88% | 11 | 100.00% | 0 | 0.00% | -5.88% | -17.07% | 5.30% | | | | | | | | | | | | |
| | Week 6 | 17 | 11 | 16 | 94.12% | 1 | 5.88% | 11 | 100.00% | 0 | 0.00% | -5.88% | -17.07% | 5.30% | | | | | | | | | | | | |
| Hair loss | Week 2 | 7 | 3 | 6 | 85.71% | 1 | 14.29% | 3 | 100.00% | 0 | 0.00% | -14.29% | -40.21% | 11.64% | n/a | | | | | | | | | | | |
| | Week 4 | 7 | 3 | 6 | 85.71% | 1 | 14.29% | 3 | 100.00% | 0 | 0.00% | -14.29% | -40.21% | 11.64% | | | | | | | | | | | | |
| | Week 6 | 7 | 3 | 7 | 100.00% | 0 | 0.00% | 3 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | |
| Other symptoms | Week 2 | 78 | 88 | 57 | 73.08% | 21 | 26.92% | 69 | 78.41% | 19 | 21.59% | -5.33% | -18.40% | 7.74% | 0.68 | 0.43 | 1.07 | 0.33 | 2 | 0.849 | 2.46 | 2 | 0.116 | 0.470 | 0.207 | 0.535 |
| | Week 4 | 78 | 88 | 62 | 79.49% | 16 | 20.51% | 77 | 87.50% | 11 | 12.50% | -8.01% | -19.33% | 3.30% | | | | | | | | | | | | |
| | Week 6 | 78 | 88 | 63 | 80.77% | 15 | 19.23% | 75 | 85.23% | 13 | 14.77% | -4.46% | -15.92% | 7.01% | | | | | | | | | | | | |

CI, confidence interval; LCL, lower confidence limit; n, number of patients with non-missing values used for the respective analysis; n/a, not applicable; OR, odds ratio; UCL, upper confidence limit.

* Adjusted P values (see section 6).

Supplementary Table 9: Effects of nicotinamide on COVID-19 symptoms (change to baseline) in males of the RFITT population (binary symptoms).

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|--------------------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|-------|------------------------------|----|-------|------------------------------|--------|--------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | χ2MH | df | P | Week 2 | Week 4 | Week 6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Performance drop | Week 2 | 73 | 77 | 48 | 65.75% | 25 | 34.25% | 34 | 44.16% | 43 | 55.84% | 21.60% | 6.06% | 37.14% | 2.43 | 1.25 | 4.70 | 4.30 | 2 | 0.038 | 0.009 | 0.861 | 0.232 |
| | Week 4 | 73 | 77 | 50 | 68.49% | 23 | 31.51% | 54 | 70.13% | 23 | 29.87% | -1.64% | -16.40% | 13.13% | 0.93 | 0.46 | 1.85 | | | | | | |
| | Week 6 | 73 | 77 | 61 | 83.56% | 12 | 16.44% | 58 | 75.32% | 19 | 24.68% | 8.24% | -4.61% | 21.08% | 1.67 | 0.74 | 3.73 | | | | | | |
| Fatigue | Week 2 | 82 | 78 | 48 | 58.54% | 34 | 41.46% | 45 | 57.69% | 33 | 42.31% | 0.84% | -14.45% | 16.14% | 1.04 | 0.55 | 1.94 | 0.00 | 2 | 0.989 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 82 | 78 | 65 | 79.27% | 17 | 20.73% | 62 | 79.49% | 16 | 20.51% | -0.22% | -12.76% | 12.32% | 0.99 | 0.46 | 2.12 | | | | | | |
| | Week 6 | 82 | 78 | 69 | 84.15% | 13 | 15.85% | 66 | 84.62% | 12 | 15.38% | -0.47% | -11.72% | 10.78% | 0.97 | 0.41 | 2.27 | | | | | | |
| Fever | Week 2 | 15 | 16 | 15 | 100.00% | 0 | 0.00% | 16 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 15 | 16 | 15 | 100.00% | 0 | 0.00% | 16 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 15 | 16 | 15 | 100.00% | 0 | 0.00% | 16 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Chills | Week 2 | 12 | 7 | 12 | 100.00% | 0 | 0.00% | 7 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 12 | 7 | 12 | 100.00% | 0 | 0.00% | 7 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 12 | 7 | 12 | 100.00% | 0 | 0.00% | 7 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Shortness of breath | Week 2 | 36 | 28 | 24 | 66.67% | 12 | 33.33% | 14 | 50.00% | 14 | 50.00% | 16.67% | -7.42% | 40.75% | 2.00 | 0.73 | 5.52 | 0.16 | 2 | 0.693 | 0.207 | 0.788 | 1.000 |
| | Week 4 | 36 | 28 | 24 | 66.67% | 12 | 33.33% | 20 | 71.43% | 8 | 28.57% | -4.76% | -27.50% | 17.98% | 0.80 | 0.27 | 2.34 | | | | | | |
| | Week 6 | 36 | 28 | 28 | 77.78% | 8 | 22.22% | 22 | 78.57% | 6 | 21.43% | -0.79% | -21.18% | 19.59% | 0.95 | 0.29 | 3.16 | | | | | | |
| Whistling / Wheezing breathing | Week 2 | 16 | 14 | 15 | 93.75% | 1 | 6.25% | 13 | 92.86% | 1 | 7.14% | 0.89% | -17.07% | 18.86% | 1.15 | 0.07 | 20.34 | 0.41 | 2 | 0.522 | 1.000 | 0.467 | 0.467 |
| | Week 4 | 16 | 14 | 16 | 100.00% | 0 | 0.00% | 13 | 92.86% | 1 | 7.14% | 7.14% | -6.35% | 20.63% | 2.46 | 0.08 | 79.33 | | | | | | |
| | Week 6 | 16 | 14 | 16 | 100.00% | 0 | 0.00% | 13 | 92.86% | 1 | 7.14% | 7.14% | -6.35% | 20.63% | 2.46 | 0.08 | 79.33 | | | | | | |
| Cough | Week 2 | 40 | 47 | 29 | 72.50% | 11 | 27.50% | 35 | 74.47% | 12 | 25.53% | -1.97% | -20.59% | 16.66% | 0.90 | 0.35 | 2.35 | 0.01 | 2 | 0.928 | 1.000 | 0.562 | 0.721 |
| | Week 4 | 40 | 47 | 33 | 82.50% | 7 | 17.50% | 41 | 87.23% | 6 | 12.77% | -4.73% | -19.89% | 10.42% | 0.69 | 0.21 | 2.25 | | | | | | |
| | Week 6 | 40 | 47 | 37 | 92.50% | 3 | 7.50% | 42 | 89.36% | 5 | 10.64% | 3.14% | -8.88% | 15.15% | 1.47 | 0.33 | 6.57 | | | | | | |
| Cough with sputum production | Week 2 | 40 | 44 | 29 | 72.50% | 11 | 27.50% | 37 | 84.09% | 7 | 15.91% | -11.59% | -29.15% | 5.97% | 0.50 | 0.17 | 1.45 | 2.47 | 2 | 0.11 | 0.287 | 0.418 | 0.476 |
| | Week 4 | 40 | 44 | 36 | 90.00% | 4 | 10.00% | 42 | 95.45% | 2 | 4.55% | -5.45% | -16.60% | 5.70% | 0.43 | 0.07 | 2.48 | | | | | | |
| | Week 6 | 40 | 44 | 39 | 97.50% | 1 | 2.50% | 44 | 100.00% | 0 | 0.00% | -2.50% | -7.34% | 2.34% | 0.44 | 0.01 | 13.58 | | | | | | |

Supplement to Schreiber S, Waetzig GH, *et al.*
Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|---------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|-------|------------------------------|----|-------|------------------------------|--------|--------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | χ2MH | df | P | Week 2 | Week 4 | Week 6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Rhinitis/rhinorrhea | Week 2 | 73 | 78 | 57 | 78.08% | 16 | 21.92% | 58 | 74.36% | 20 | 25.64% | 3.72% | -9.84% | 17.29% | 1.23 | 0.58 | 2.61 | 2.18 | 2 | 0.139 | 0.703 | 0.189 | 0.368 |
| | Week 4 | 73 | 78 | 68 | 93.15% | 5 | 6.85% | 67 | 85.90% | 11 | 14.10% | 7.25% | -2.40% | 16.91% | 2.23 | 0.74 | 6.77 | | | | | | |
| | Week 6 | 73 | 78 | 72 | 98.63% | 1 | 1.37% | 74 | 94.87% | 4 | 5.13% | 3.76% | -1.82% | 9.33% | 3.89 | 0.42 | 35.66 | | | | | | |
| Sore throat | Week 2 | 35 | 39 | 32 | 91.43% | 3 | 8.57% | 36 | 92.31% | 3 | 7.69% | -0.88% | -13.37% | 11.61% | 0.89 | 0.17 | 4.72 | 0.04 | 2 | 0.847 | 1.000 | 0.662 | 1.000 |
| | Week 4 | 35 | 39 | 32 | 91.43% | 3 | 8.57% | 37 | 94.87% | 2 | 5.13% | -3.44% | -15.02% | 8.13% | 0.58 | 0.09 | 3.67 | | | | | | |
| | Week 6 | 35 | 39 | 35 | 100.00% | 0 | 0.00% | 38 | 97.44% | 1 | 2.56% | 2.56% | -2.40% | 7.52% | 1.84 | 0.06 | 56.64 | | | | | | |
| Hoarseness | Week 2 | 31 | 31 | 29 | 93.55% | 2 | 6.45% | 31 | 100.00% | 0 | 0.00% | -6.45% | -15.10% | 2.20% | 0.23 | 0.01 | 5.40 | 0.25 | 2 | 0.616 | 0.492 | 1.000 | 1.000 |
| | Week 4 | 31 | 31 | 31 | 100.00% | 0 | 0.00% | 30 | 96.77% | 1 | 3.23% | 3.23% | -2.99% | 9.45% | 2.07 | 0.07 | 63.92 | | | | | | |
| | Week 6 | 31 | 31 | 30 | 96.77% | 1 | 3.23% | 31 | 100.00% | 0 | 0.00% | -3.23% | -9.45% | 2.99% | 0.48 | 0.02 | 14.97 | | | | | | |
| Pneumonia | Week 2 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Muscle pain | Week 2 | 37 | 30 | 37 | 100.00% | 0 | 0.00% | 30 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 37 | 30 | 37 | 100.00% | 0 | 0.00% | 29 | 96.67% | 1 | 3.33% | 3.33% | -3.09% | 9.76% | 2.55 | 0.08 | 78.74 | | | | | | |
| | Week 6 | 37 | 30 | 37 | 100.00% | 0 | 0.00% | 30 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Joint pain | Week 2 | 24 | 28 | 23 | 95.83% | 1 | 4.17% | 27 | 96.43% | 1 | 3.57% | -0.60% | -11.14% | 9.95% | 0.85 | 0.05 | 14.39 | 0.02 | 2 | 0.893 | 1.000 | 1.000 | 0.462 |
| | Week 4 | 24 | 28 | 24 | 100.00% | 0 | 0.00% | 28 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | n/a | | | | | | | | |
| | Week 6 | 24 | 28 | 23 | 95.83% | 1 | 4.17% | 28 | 100.00% | 0 | 0.00% | -4.17% | -12.16% | 3.83% | 0.41 | 0.01 | 12.80 | | | | | | |
| Limb pain | Week 2 | 32 | 35 | 29 | 90.63% | 3 | 9.38% | 35 | 100.00% | 0 | 0.00% | -9.38% | -19.47% | 0.72% | 0.14 | 0.01 | 2.87 | 1.57 | 2 | 0.210 | 0.104 | 1.000 | 1.000 |
| | Week 4 | 32 | 35 | 32 | 100.00% | 0 | 0.00% | 35 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 32 | 35 | 32 | 100.00% | 0 | 0.00% | 35 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Chest pain | Week 2 | 28 | 16 | 25 | 89.29% | 3 | 10.71% | 16 | 100.00% | 0 | 0.00% | -10.71% | -22.17% | 0.74% | 0.26 | 0.01 | 5.55 | 0.70 | 2 | 0.404 | 0.290 | 0.526 | 1.000 |
| | Week 4 | 28 | 16 | 26 | 92.86% | 2 | 7.14% | 16 | 100.00% | 0 | 0.00% | -7.14% | -16.68% | 2.40% | 0.41 | 0.02 | 9.59 | | | | | | |
| | Week 6 | 28 | 16 | 27 | 96.43% | 1 | 3.57% | 15 | 93.75% | 1 | 6.25% | 2.68% | -11.03% | 16.39% | 1.80 | 0.10 | 30.90 | | | | | | |
| Headache | Week 2 | 47 | 43 | 36 | 76.60% | 11 | 23.40% | 35 | 81.40% | 8 | 18.60% | -4.80% | -21.59% | 11.99% | 0.75 | 0.27 | 2.08 | 0.00 | 2 | 0.947 | 0.615 | 0.705 | 0.345 |
| | Week 4 | 47 | 43 | 44 | 93.62% | 3 | 6.38% | 39 | 90.70% | 4 | 9.30% | 2.92% | -8.23% | 14.06% | 1.50 | 0.32 | 7.14 | | | | | | |
| | Week 6 | 47 | 43 | 46 | 97.87% | 1 | 2.13% | 40 | 93.02% | 3 | 6.98% | 4.85% | -3.81% | 13.51% | 3.45 | 0.35 | 34.50 | | | | | | |

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Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|--------------------------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|-------|------------------------------|----|-------|------------------------------|--------|--------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | χ2MH | df | P | Week 2 | Week 4 | Week 6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Abdominal pain | Week 2 | 9 | 6 | 9 | 100.00% | 0 | 0.00% | 6 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | n/a | | | | | | | | |
| | Week 4 | 9 | 6 | 9 | 100.00% | 0 | 0.00% | 6 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | n/a | | | | | | | | |
| | Week 6 | 9 | 6 | 9 | 100.00% | 0 | 0.00% | 6 | 100.00% | 0 | 0.00% | 0.00% | 0.00% | 0.00% | n/a | | | | | | | | |
| Diarrhea | Week 2 | 10 | 20 | 10 | 100.00% | 0 | 0.00% | 18 | 90.00% | 2 | 10.00% | 10.00% | -3.15% | 23.15% | 2.22 | 0.09 | 54.19 | 0.06 | 2 | 0.799 | 0.540 | 1.000 | 1.000 |
| | Week 4 | 10 | 20 | 10 | 100.00% | 0 | 0.00% | 20 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 10 | 20 | 10 | 100.00% | 0 | 0.00% | 20 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Nausea | Week 2 | 8 | 8 | 8 | 100.00% | 0 | 0.00% | 8 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 8 | 8 | 8 | 100.00% | 0 | 0.00% | 8 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 8 | 8 | 8 | 100.00% | 0 | 0.00% | 8 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Vomiting | Week 2 | 0 | 1 | 0 | 0.00% | 0 | 0.00% | 1 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 0 | 1 | 0 | 0.00% | 0 | 0.00% | 1 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 0 | 1 | 0 | 0.00% | 0 | 0.00% | 1 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Loss of appetite / lower food intake | Week 2 | 47 | 45 | 45 | 95.74% | 2 | 4.26% | 43 | 95.56% | 2 | 4.44% | 0.19% | -8.15% | 8.53% | 1.05 | 0.14 | 7.76 | 0.07 | 2 | 0.786 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 47 | 45 | 45 | 95.74% | 2 | 4.26% | 44 | 97.78% | 1 | 2.22% | -2.03% | -9.23% | 5.17% | 0.51 | 0.04 | 5.84 | | | | | | |
| | Week 6 | 47 | 45 | 46 | 97.87% | 1 | 2.13% | 44 | 97.78% | 1 | 2.22% | 0.09% | -5.87% | 6.06% | 1.05 | 0.06 | 17.24 | | | | | | |
| Other gastrointes- tinal symptoms | Week 2 | 5 | 2 | 4 | 80.00% | 1 | 20.00% | 2 | 100.00% | 0 | 0.00% | -20.00% | -55.06% | 15.06% | 1.00 | 0.02 | 44.50 | 0.40 | 2 | 0.527 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 5 | 2 | 5 | 100.00% | 0 | 0.00% | 2 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 5 | 2 | 5 | 100.00% | 0 | 0.00% | 2 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Impaired sense of smell | Week 2 | 45 | 57 | 21 | 46.67% | 24 | 53.33% | 27 | 47.37% | 30 | 52.63% | -0.70% | -20.21% | 18.80% | 0.97 | 0.44 | 2.13 | 0.16 | 2 | 0.687 | 1.000 | 0.394 | 1.000 |
| | Week 4 | 45 | 57 | 28 | 62.22% | 17 | 37.78% | 41 | 71.93% | 16 | 28.07% | -9.71% | -28.06% | 8.64% | 0.64 | 0.28 | 1.48 | | | | | | |
| | Week 6 | 45 | 57 | 34 | 75.56% | 11 | 24.44% | 42 | 73.68% | 15 | 26.32% | 1.87% | -15.11% | 18.85% | 1.10 | 0.45 | 2.71 | | | | | | |
| Impaired sense of taste | Week 2 | 44 | 56 | 23 | 52.27% | 21 | 47.73% | 33 | 58.93% | 23 | 41.07% | -6.66% | -26.25% | 12.94% | 0.76 | 0.34 | 1.69 | 0.27 | 2 | 0.604 | 0.547 | 0.505 | 0.803 |
| | Week 4 | 44 | 56 | 30 | 68.18% | 14 | 31.82% | 42 | 75.00% | 14 | 25.00% | -6.82% | -24.65% | 11.02% | 0.71 | 0.30 | 1.72 | | | | | | |
| | Week 6 | 44 | 56 | 36 | 81.82% | 8 | 18.18% | 44 | 78.57% | 12 | 21.43% | 3.25% | -12.42% | 18.91% | 1.23 | 0.45 | 3.33 | | | | | | |
| Confusion | Week 2 | 7 | 8 | 6 | 85.71% | 1 | 14.29% | 8 | 100.00% | 0 | 0.00% | -14.29% | -40.21% | 11.64% | 0.38 | 0.01 | 13.13 | 0.01 | 2 | 0.925 | 0.467 | 1.000 | 1.000 |
| | Week 4 | 7 | 8 | 7 | 100.00% | 0 | 0.00% | 7 | 87.50% | 1 | 12.50% | 12.50% | -10.42% | 35.42% | 2.00 | 0.06 | 69.82 | | | | | | |
| | Week 6 | 7 | 8 | 7 | 100.00% | 0 | 0.00% | 8 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |

Supplement to Schreiber S, Waetzig GH, *et al.*
Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|----------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|--------|------------------------------|----|--------|------------------------------|--------|--------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | χ2MH | df | P | Week 2 | Week 4 | Week 6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Dizziness | Week 2 | 17 | 9 | 17 | 100.00% | 0 | 0.00% | 7 | 77.78% | 2 | 22.22% | 22.22% | -4.94% | 49.38% | 9.71 | 0.39 | 243.52 | 0.78 | 2 | 0.378 | 0.111 | 1.000 | 1.000 |
| | Week 4 | 17 | 9 | 16 | 94.12% | 1 | 5.88% | 8 | 88.89% | 1 | 11.11% | 5.23% | -18.15% | 28.61% | 2.00 | 0.11 | 36.31 | | | | | | |
| | Week 6 | 17 | 9 | 15 | 88.24% | 2 | 11.76% | 8 | 88.89% | 1 | 11.11% | -0.65% | -26.27% | 24.96% | 0.94 | 0.07 | 12.00 | | | | | | |
| Conjunctivitis | Week 2 | 1 | 4 | 1 | 100.00% | 0 | 0.00% | 4 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 1 | 4 | 1 | 100.00% | 0 | 0.00% | 4 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 1 | 4 | 1 | 100.00% | 0 | 0.00% | 4 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Skin rash | Week 2 | 7 | 3 | 6 | 85.71% | 1 | 14.29% | 3 | 100.00% | 0 | 0.00% | -14.29% | -40.21% | 11.64% | 1.00 | 0.03 | 39.13 | 0.02 | 2 | 0.8774 | 1.0000 | 1.0000 | 1.0000 |
| | Week 4 | 7 | 3 | 6 | 85.71% | 1 | 14.29% | 3 | 100.00% | 0 | 0.00% | -14.29% | -40.21% | 11.64% | 1.00 | 0.03 | 39.13 | | | | | | |
| | Week 6 | 7 | 3 | 7 | 100.00% | 0 | 0.00% | 3 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Hair loss | Week 2 | 1 | 0 | 1 | 100.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 1 | 0 | 1 | 100.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 1 | 0 | 1 | 100.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Other symptoms | Week 2 | 34 | 36 | 29 | 85.29% | 5 | 14.71% | 29 | 80.56% | 7 | 19.44% | 4.74% | -12.84% | 22.31% | 1.40 | 0.40 | 4.92 | 0.09 | 2 | 0.766 | 0.754 | 0.669 | 0.674 |
| | Week 4 | 34 | 36 | 31 | 91.18% | 3 | 8.82% | 34 | 94.44% | 2 | 5.56% | -3.27% | -15.39% | 8.85% | 0.61 | 0.10 | 3.88 | | | | | | |
| | Week 6 | 34 | 36 | 32 | 94.12% | 2 | 5.88% | 32 | 88.89% | 4 | 11.11% | 5.23% | -7.73% | 18.19% | 2.00 | 0.34 | 11.70 | | | | | | |

CI, confidence interval; LCL, lower confidence limit; n, number of patients with non-missing values used for the respective analysis; n/a, not applicable; OR, odds ratio; UCL, upper confidence limit.

* Adjusted P values (see section 6).

Supplementary Table 10: Effects of nicotinamide on COVID-19 symptoms (change to baseline) in females of the RFITT population (binary symptoms).

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|--------------------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|------|------------------------------|----|-------|------------------------------|-------|-------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | χ^2 MH | df | P | Week2 | Week4 | Week6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Performance drop | Week 2 | 118 | 111 | 62 | 52.54% | 56 | 47.46% | 46 | 41.44% | 65 | 58.56% | 11.10% | -1.75% | 23.95% | 1.56 | 0.93 | 2.64 | 1.28 | 2 | 0.257 | 0.112 | 0.784 | 1.000 |
| | Week 4 | 118 | 111 | 76 | 64.41% | 42 | 35.59% | 69 | 62.16% | 42 | 37.84% | 2.24% | -10.25% | 14.74% | 1.10 | 0.64 | 1.89 | | | | | | |
| | Week 6 | 118 | 111 | 87 | 73.73% | 31 | 26.27% | 82 | 73.87% | 29 | 26.13% | -0.15% | -11.54% | 11.25% | 0.99 | 0.55 | 1.79 | | | | | | |
| Fatigue | Week 2 | 117 | 120 | 57 | 48.72% | 60 | 51.28% | 51 | 42.50% | 69 | 57.50% | 6.22% | -6.44% | 18.88% | 1.29 | 0.77 | 2.15 | 0.09 | 2 | 0.764 | 0.363 | 0.679 | 0.442 |
| | Week 4 | 117 | 120 | 81 | 69.23% | 36 | 30.77% | 80 | 66.67% | 40 | 33.33% | 2.56% | -9.31% | 14.44% | 1.13 | 0.65 | 1.94 | | | | | | |
| | Week 6 | 117 | 120 | 87 | 74.36% | 30 | 25.64% | 95 | 79.17% | 25 | 20.83% | -4.81% | -15.55% | 5.93% | 0.76 | 0.42 | 1.40 | | | | | | |
| Fever | Week 2 | 10 | 21 | 10 | 100.00% | 0 | 0.00% | 21 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 10 | 21 | 10 | 100.00% | 0 | 0.00% | 21 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 10 | 21 | 10 | 100.00% | 0 | 0.00% | 21 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Chills | Week 2 | 12 | 22 | 12 | 100.00% | 0 | 0.00% | 22 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 12 | 22 | 12 | 100.00% | 0 | 0.00% | 22 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 12 | 22 | 12 | 100.00% | 0 | 0.00% | 22 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Shortness of breath | Week 2 | 56 | 62 | 32 | 57.14% | 24 | 42.86% | 23 | 37.10% | 39 | 62.90% | 20.05% | 2.37% | 37.73% | 2.26 | 1.08 | 4.73 | 0.27 | 2 | 0.602 | 0.042 | 0.851 | 0.420 |
| | Week 4 | 56 | 62 | 34 | 60.71% | 22 | 39.29% | 39 | 62.90% | 23 | 37.10% | -2.19% | -19.74% | 15.37% | 0.91 | 0.43 | 1.92 | | | | | | |
| | Week 6 | 56 | 62 | 37 | 66.07% | 19 | 33.93% | 46 | 74.19% | 16 | 25.81% | -8.12% | -24.63% | 8.38% | 0.68 | 0.31 | 1.50 | | | | | | |
| Whistling / Wheezing breathing | Week 2 | 12 | 20 | 10 | 83.33% | 2 | 16.67% | 19 | 95.00% | 1 | 5.00% | -11.67% | -34.82% | 11.48% | 0.26 | 0.02 | 3.27 | 0.21 | 2 | 0.644 | 0.540 | 1.000 | 1.000 |
| | Week 4 | 12 | 20 | 12 | 100.00% | 0 | 0.00% | 20 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 12 | 20 | 12 | 100.00% | 0 | 0.00% | 20 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Cough | Week 2 | 76 | 72 | 55 | 72.37% | 21 | 27.63% | 45 | 62.50% | 27 | 37.50% | 9.87% | -5.17% | 24.91% | 1.57 | 0.79 | 3.14 | 0.02 | 2 | 0.881 | 0.222 | 0.270 | 1.000 |
| | Week 4 | 76 | 72 | 61 | 80.26% | 15 | 19.74% | 63 | 87.50% | 9 | 12.50% | -7.24% | -19.00% | 4.53% | 0.58 | 0.24 | 1.43 | | | | | | |
| | Week 6 | 76 | 72 | 72 | 94.74% | 4 | 5.26% | 68 | 94.44% | 4 | 5.56% | 0.29% | -7.00% | 7.59% | 1.06 | 0.25 | 4.40 | | | | | | |
| Cough with sputum production | Week 2 | 53 | 51 | 43 | 81.13% | 10 | 18.87% | 37 | 72.55% | 14 | 27.45% | 8.58% | -7.57% | 24.74% | 1.63 | 0.65 | 4.09 | 0.00 | 2 | 0.980 | 0.356 | 0.150 | 1.000 |
| | Week 4 | 53 | 51 | 43 | 81.13% | 10 | 18.87% | 47 | 92.16% | 4 | 7.84% | -11.02% | -23.89% | 1.84% | 0.37 | 0.11 | 1.25 | | | | | | |
| | Week 6 | 53 | 51 | 50 | 94.34% | 3 | 5.66% | 48 | 94.12% | 3 | 5.88% | 0.22% | -8.75% | 9.19% | 1.04 | 0.20 | 5.42 | | | | | | |

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Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|---------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|-------|------------------------------|----|-------|------------------------------|-------|-------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | χ2MH | df | P | Week2 | Week4 | Week6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Rhinitis/rhinorrhea | Week 2 | 111 | 100 | 86 | 77.48% | 25 | 22.52% | 68 | 68.00% | 32 | 32.00% | 9.48% | -2.52% | 21.48% | 1.62 | 0.88 | 2.99 | 2.96 | 2 | 0.085 | 0.162 | 0.266 | 1.000 |
| | Week 4 | 111 | 100 | 96 | 86.49% | 15 | 13.51% | 80 | 80.00% | 20 | 20.00% | 6.49% | -3.61% | 16.58% | 1.60 | 0.77 | 3.33 | | | | | | |
| | Week 6 | 111 | 100 | 107 | 96.40% | 4 | 3.60% | 97 | 97.00% | 3 | 3.00% | -0.60% | -5.42% | 4.21% | 0.83 | 0.18 | 3.79 | | | | | | |
| Sore throat | Week 2 | 43 | 54 | 40 | 93.02% | 3 | 6.98% | 45 | 83.33% | 9 | 16.67% | 9.69% | -2.83% | 22.21% | 2.67 | 0.67 | 10.54 | 0.56 | 2 | 0.453 | 0.217 | 1.000 | 0.443 |
| | Week 4 | 43 | 54 | 39 | 90.70% | 4 | 9.30% | 48 | 88.89% | 6 | 11.11% | 1.81% | -10.26% | 13.88% | 1.22 | 0.32 | 4.63 | | | | | | |
| | Week 6 | 43 | 54 | 42 | 97.67% | 1 | 2.33% | 54 | 100.00% | 0 | 0.00% | -2.33% | -6.83% | 2.18% | 0.39 | 0.01 | 11.87 | | | | | | |
| Hoarseness | Week 2 | 45 | 61 | 38 | 84.44% | 7 | 15.56% | 55 | 90.16% | 0 | 0.00% | -5.72% | -18.68% | 7.24% | 0.59 | 0.18 | 1.90 | 0.00 | 2 | 0.974 | 0.388 | 0.238 | 1.000 |
| | Week 4 | 45 | 61 | 44 | 97.78% | 1 | 2.22% | 56 | 91.80% | 0 | 0.00% | 5.97% | -2.15% | 14.09% | 3.93 | 0.44 | 34.86 | | | | | | |
| | Week 6 | 45 | 61 | 45 | 100.00% | 0 | 0.00% | 61 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Pneumonia | Week 2 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 0 | 0 | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Muscle pain | Week 2 | 42 | 53 | 40 | 95.24% | 2 | 4.76% | 51 | 96.23% | 2 | 3.77% | -0.99% | -9.22% | 7.25% | 0.78 | 0.11 | 5.81 | 0.19 | 2 | 0.667 | 1.000 | 0.696 | 1.000 |
| | Week 4 | 42 | 53 | 38 | 90.48% | 4 | 9.52% | 50 | 94.34% | 3 | 5.66% | -3.86% | -14.70% | 6.98% | 0.57 | 0.12 | 2.70 | | | | | | |
| | Week 6 | 42 | 53 | 41 | 97.62% | 1 | 2.38% | 52 | 98.11% | 1 | 1.89% | -0.49% | -6.38% | 5.39% | 0.79 | 0.05 | 12.99 | | | | | | |
| Joint pain | Week 2 | 33 | 27 | 30 | 90.91% | 3 | 9.09% | 24 | 88.89% | 3 | 11.11% | 2.02% | -13.37% | 17.41% | 1.25 | 0.23 | 6.76 | 0.60 | 2 | 0.437 | 1.000 | 0.620 | 0.245 |
| | Week 4 | 33 | 27 | 30 | 90.91% | 3 | 9.09% | 26 | 96.30% | 1 | 3.70% | -5.39% | -17.51% | 6.74% | 0.38 | 0.04 | 3.93 | | | | | | |
| | Week 6 | 33 | 27 | 30 | 90.91% | 3 | 9.09% | 27 | 100.00% | 0 | 0.00% | -9.09% | -18.90% | 0.72% | 0.19 | 0.01 | 3.87 | | | | | | |
| Limb pain | Week 2 | 39 | 52 | 37 | 94.87% | 2 | 5.13% | 49 | 94.23% | 3 | 5.77% | 0.64% | -8.74% | 10.03% | 1.13 | 0.18 | 7.13 | 0.15 | 2 | 0.698 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 39 | 52 | 39 | 100.00% | 0 | 0.00% | 51 | 98.08% | 1 | 1.92% | 1.92% | -1.81% | 5.66% | 1.53 | 0.05 | 46.77 | | | | | | |
| | Week 6 | 39 | 52 | 39 | 100.00% | 0 | 0.00% | 51 | 98.08% | 1 | 1.92% | 1.92% | -1.81% | 5.66% | 1.53 | 0.05 | 46.77 | | | | | | |
| Chest pain | Week 2 | 29 | 32 | 27 | 93.10% | 2 | 6.90% | 29 | 90.63% | 3 | 9.38% | 2.48% | -11.20% | 16.16% | 1.40 | 0.22 | 9.01 | 0.12 | 2 | 0.727 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 29 | 32 | 29 | 100.00% | 0 | 0.00% | 32 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 29 | 32 | 29 | 100.00% | 0 | 0.00% | 32 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Headache | Week 2 | 66 | 72 | 54 | 81.82% | 12 | 18.18% | 50 | 69.44% | 22 | 30.56% | 12.37% | -1.76% | 26.51% | 1.98 | 0.89 | 4.41 | 2.31 | 2 | 0.129 | 0.115 | 0.434 | 1.000 |
| | Week 4 | 66 | 72 | 60 | 90.91% | 6 | 9.09% | 62 | 86.11% | 10 | 13.89% | 4.80% | -5.78% | 15.38% | 1.61 | 0.55 | 4.71 | | | | | | |
| | Week 6 | 66 | 72 | 62 | 93.94% | 4 | 6.06% | 68 | 94.44% | 4 | 5.56% | -0.51% | -8.32% | 7.31% | 0.91 | 0.22 | 3.80 | | | | | | |

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Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|--------------------------------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|---------|--------|------------|--------|--------|------------------------------|----|-------|------------------------------|-------|-------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | χ2MH | df | P | Week2 | Week4 | Week6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Abdominal pain | Week 2 | 10 | 15 | 9 | 90.00% | 1 | 10.00% | 13 | 86.67% | 2 | 13.33% | 3.33% | -22.00% | 28.66% | 1.38 | 0.11 | 17.67 | 0.00 | 2 | 1.000 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 10 | 15 | 10 | 100.00% | 0 | 0.00% | 15 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 10 | 15 | 9 | 90.00% | 1 | 10.00% | 14 | 93.33% | 1 | 6.67% | -3.33% | -25.81% | 19.14% | 0.64 | 0.04 | 11.63 | | | | | | |
| Diarrhea | Week 2 | 18 | 31 | 16 | 88.89% | 2 | 11.11% | 27 | 87.10% | 4 | 12.90% | 1.79% | -16.92% | 20.50% | 1.19 | 0.19 | 7.22 | 0.06 | 2 | 0.800 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 18 | 31 | 17 | 94.44% | 1 | 5.56% | 29 | 93.55% | 2 | 6.45% | 0.90% | -12.77% | 14.56% | 1.17 | 0.10 | 13.92 | | | | | | |
| | Week 6 | 18 | 31 | 17 | 94.44% | 1 | 5.56% | 29 | 93.55% | 2 | 6.45% | 0.90% | -12.77% | 14.56% | 1.17 | 0.10 | 13.92 | | | | | | |
| Nausea | Week 2 | 16 | 26 | 13 | 81.25% | 3 | 18.75% | 25 | 96.15% | 1 | 3.85% | -14.90% | -35.41% | 5.60% | 0.17 | 0.02 | 1.84 | 1.10 | 2 | 0.295 | 0.146 | 1.000 | 0.381 |
| | Week 4 | 16 | 26 | 16 | 100.00% | 0 | 0.00% | 25 | 96.15% | 1 | 3.85% | 3.85% | -3.55% | 11.24% | 1.28 | 0.04 | 40.41 | | | | | | |
| | Week 6 | 16 | 26 | 15 | 93.75% | 1 | 6.25% | 26 | 100.00% | 0 | 0.00% | -6.25% | -18.11% | 5.61% | 0.29 | 0.01 | 9.12 | | | | | | |
| Vomiting | Week 2 | 1 | 2 | 1 | 100.00% | 0 | 0.00% | 2 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 4 | 1 | 2 | 1 | 100.00% | 0 | 0.00% | 2 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 1 | 2 | 1 | 100.00% | 0 | 0.00% | 2 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Loss of appetite / lower food intake | Week 2 | 78 | 81 | 73 | 93.59% | 5 | 6.41% | 73 | 90.12% | 8 | 9.88% | 3.47% | -5.01% | 11.94% | 1.60 | 0.50 | 5.12 | 1.43 | 2 | 0.232 | 0.565 | 0.443 | 1.000 |
| | Week 4 | 78 | 81 | 76 | 97.44% | 2 | 2.56% | 76 | 93.83% | 5 | 6.17% | 3.61% | -2.70% | 9.92% | 2.50 | 0.47 | 13.29 | | | | | | |
| | Week 6 | 78 | 81 | 77 | 98.72% | 1 | 1.28% | 79 | 97.53% | 2 | 2.47% | 1.19% | -3.01% | 5.39% | 1.95 | 0.17 | 21.94 | | | | | | |
| Other gastrointes- tinal symptoms | Week 2 | 6 | 5 | 5 | 83.33% | 1 | 16.67% | 4 | 80.00% | 1 | 20.00% | 3.33% | -42.69% | 49.36% | 1.25 | 0.06 | 26.87 | 0.49 | 2 | 0.482 | 1.000 | 0.455 | 0.455 |
| | Week 4 | 6 | 5 | 6 | 100.00% | 0 | 0.00% | 4 | 80.00% | 1 | 20.00% | 20.00% | -15.06% | 55.06% | 3.00 | 0.08 | 112.34 | | | | | | |
| | Week 6 | 6 | 5 | 6 | 100.00% | 0 | 0.00% | 4 | 80.00% | 1 | 20.00% | 20.00% | -15.06% | 55.06% | 3.00 | 0.08 | 112.34 | | | | | | |
| Impaired sense of smell | Week 2 | 69 | 80 | 40 | 57.97% | 29 | 42.03% | 40 | 50.00% | 40 | 50.00% | 7.97% | -8.02% | 23.96% | 1.38 | 0.72 | 2.64 | 3.12 | 2 | 0.077 | 0.410 | 0.592 | 0.114 |
| | Week 4 | 69 | 80 | 50 | 72.46% | 19 | 27.54% | 54 | 67.50% | 26 | 32.50% | 4.96% | -9.75% | 19.68% | 1.27 | 0.63 | 2.57 | | | | | | |
| | Week 6 | 69 | 80 | 58 | 84.06% | 11 | 15.94% | 58 | 72.50% | 22 | 27.50% | 11.56% | -1.49% | 24.61% | 2.00 | 0.89 | 4.50 | | | | | | |
| Impaired sense of taste | Week 2 | 59 | 81 | 33 | 55.93% | 26 | 44.07% | 43 | 53.09% | 38 | 46.91% | 2.85% | -13.85% | 19.54% | 1.12 | 0.57 | 2.20 | 1.58 | 2 | 0.209 | 0.864 | 0.354 | 0.326 |
| | Week 4 | 59 | 81 | 44 | 74.58% | 15 | 25.42% | 54 | 66.67% | 27 | 33.33% | 7.91% | -7.22% | 23.04% | 1.47 | 0.70 | 3.09 | | | | | | |
| | Week 6 | 59 | 81 | 47 | 79.66% | 12 | 20.34% | 58 | 71.60% | 23 | 28.40% | 8.06% | -6.15% | 22.27% | 1.55 | 0.70 | 3.45 | | | | | | |
| Confusion | Week 2 | 8 | 8 | 8 | 100.00% | 0 | 0.00% | 7 | 87.50% | 1 | 12.50% | 12.50% | -10.42% | 35.42% | 2.29 | 0.07 | 79.03 | 0.00 | 2 | 1.000 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 8 | 8 | 8 | 100.00% | 0 | 0.00% | 8 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 8 | 8 | 8 | 100.00% | 0 | 0.00% | 8 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |

Supplement to Schreiber S, Waetzig GH, *et al.*
Nicotinamide for COVID-19

| Symptoms | Time | Nicotinamide | Placebo | Nicotinamide | | | | Placebo | | | | Difference | | | Odds Ratio | | | Cochran-Mantel-Haenszel test | | | Two-sided Fisher exact test* | | |
|----------------|--------|--------------|---------|-------------------------|---------|--------------------|--------|-------------------------|---------|--------------------|--------|------------|----------|--------|------------|--------|-------|------------------------------|----|-------|------------------------------|-------|-------|
| | | n total | n total | Resolution vs. baseline | | Persisting symptom | | Resolution vs. baseline | | Persisting symptom | | Diff. | 95% CI | | OR | 95% CI | | χ^2 MH | df | P | Week2 | Week4 | Week6 |
| | | | | n | % | n | % | n | % | n | % | | LCL | UCL | | LCL | UCL | | | | P | P | P |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Dizziness | Week 2 | 33 | 40 | 30 | 90.91% | 3 | 9.09% | 33 | 82.50% | 7 | 17.50% | 8.41% | -6.92% | 23.73% | 2.12 | 0.50 | 8.95 | 0.02 | 2 | 0.879 | 0.496 | 1.000 | 0.586 |
| | Week 4 | 33 | 40 | 32 | 96.97% | 1 | 3.03% | 39 | 97.50% | 1 | 2.50% | -0.53% | -8.12% | 7.06% | 0.82 | 0.05 | 13.64 | | | | | | |
| | Week 6 | 33 | 40 | 31 | 93.94% | 2 | 6.06% | 39 | 97.50% | 1 | 2.50% | -3.56% | -13.03% | 5.91% | 0.40 | 0.03 | 4.59 | | | | | | |
| Conjunctivitis | Week 2 | 2 | 5 | 1 | 50.00% | 1 | 50.00% | 5 | 100.00% | 0 | 0.00% | -50.00% | -119.30% | 19.30% | 0.10 | 0.00 | 5.55 | 0.45 | 2 | 0.502 | 0.286 | 1.000 | 1.000 |
| | Week 4 | 2 | 5 | 2 | 100.00% | 0 | 0.00% | 4 | 80.00% | 1 | 20.00% | 20.00% | -15.06% | 55.06% | 1.00 | 0.02 | 44.50 | | | | | | |
| | Week 6 | 2 | 5 | 2 | 100.00% | 0 | 0.00% | 5 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Skin rash | Week 2 | 10 | 8 | 9 | 90.00% | 1 | 10.00% | 8 | 100.00% | 0 | 0.00% | -10.00% | -28.59% | 8.59% | 0.56 | 0.02 | 19.12 | 0.31 | 2 | 0.580 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 10 | 8 | 10 | 100.00% | 0 | 0.00% | 8 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| | Week 6 | 10 | 8 | 9 | 90.00% | 1 | 10.00% | 8 | 100.00% | 0 | 0.00% | -10.00% | -28.59% | 8.59% | 0.56 | 0.02 | 19.12 | | | | | | |
| Hair loss | Week 2 | 6 | 3 | 5 | 83.33% | 1 | 16.67% | 3 | 100.00% | 0 | 0.00% | -16.67% | -46.49% | 13.15% | 0.83 | 0.02 | 33.18 | 0.06 | 2 | 0.803 | 1.000 | 1.000 | 1.000 |
| | Week 4 | 6 | 3 | 5 | 83.33% | 1 | 16.67% | 3 | 100.00% | 0 | 0.00% | -16.67% | -46.49% | 13.15% | 0.83 | 0.02 | 33.18 | | | | | | |
| | Week 6 | 6 | 3 | 6 | 100.00% | 0 | 0.00% | 3 | 100.00% | 0 | 0.00% | n/a | | | | | | | | | | | |
| Other symptoms | Week 2 | 44 | 52 | 28 | 63.64% | 16 | 36.36% | 40 | 76.92% | 12 | 23.08% | -13.29% | -31.54% | 4.97% | 0.53 | 0.22 | 1.28 | 5.36 | 2 | 0.021 | 0.181 | 0.223 | 0.223 |
| | Week 4 | 44 | 52 | 31 | 70.45% | 13 | 29.55% | 43 | 82.69% | 9 | 17.31% | -12.24% | -29.19% | 4.72% | 0.50 | 0.19 | 1.31 | | | | | | |
| | Week 6 | 44 | 52 | 31 | 70.45% | 13 | 29.55% | 43 | 82.69% | 9 | 17.31% | -12.24% | -29.19% | 4.72% | 0.50 | 0.19 | 1.31 | | | | | | |

CI, confidence interval; LCL, lower confidence limit; n, number of patients with non-missing values used for the respective analysis; n/a, not applicable; OR, odds ratio; UCL, upper confidence limit.

* Adjusted P values (see section 6).

Supplementary Table 11: Effects of nicotinamide on COVID-19 symptoms (change to baseline) in the entire RFITT population (complaint scale).

| Characteristics / symptoms | Time | Nicotinamide | | | Placebo | | | Contrast | 95% CI | | T | df | P |
|--------------------------------------|--------|--------------|-------|------|---------|-------|------|----------|--------|-------|-------|--------|-------|
| | | n | Mean | SE | n | Mean | SE | | LCL | UCL | | | |
| Ability to perform normal activities | Week 2 | 103 | -3.07 | 0.12 | 95 | -2.62 | 0.13 | -0.45 | -0.80 | -0.11 | -2.62 | 519.79 | 0.009 |
| | Week 4 | 103 | -3.49 | 0.12 | 95 | -3.34 | 0.13 | -0.15 | -0.49 | 0.20 | -0.84 | 519.79 | 0.400 |
| | Week 6 | 103 | -3.87 | 0.12 | 95 | -3.92 | 0.13 | 0.05 | -0.29 | 0.40 | 0.31 | 519.79 | 0.758 |
| Cough | Week 2 | 44 | -3.22 | 0.16 | 33 | -2.76 | 0.18 | -0.46 | -0.94 | 0.02 | -1.91 | 249.68 | 0.057 |
| | Week 4 | 44 | -3.43 | 0.16 | 33 | -3.43 | 0.18 | 0.00 | -0.48 | 0.48 | 0.00 | 249.68 | 0.997 |
| | Week 6 | 44 | -4.07 | 0.16 | 33 | -4.01 | 0.18 | -0.06 | -0.54 | 0.42 | -0.25 | 249.68 | 0.805 |
| Mucus production | Week 2 | 40 | -3.43 | 0.16 | 25 | -3.15 | 0.20 | -0.28 | -0.78 | 0.22 | -1.09 | 224.75 | 0.276 |
| | Week 4 | 40 | -3.61 | 0.16 | 25 | -3.55 | 0.20 | -0.05 | -0.56 | 0.45 | -0.21 | 224.75 | 0.834 |
| | Week 6 | 40 | -4.08 | 0.16 | 25 | -4.03 | 0.20 | -0.05 | -0.55 | 0.45 | -0.19 | 224.75 | 0.850 |
| Shortness of breath | Week 2 | 20 | -2.23 | 0.35 | 27 | -2.20 | 0.30 | -0.02 | -0.94 | 0.89 | -0.05 | 123.64 | 0.959 |
| | Week 4 | 20 | -2.78 | 0.35 | 27 | -2.61 | 0.30 | -0.17 | -1.08 | 0.74 | -0.36 | 123.64 | 0.718 |
| | Week 6 | 20 | -3.33 | 0.35 | 27 | -2.98 | 0.30 | -0.35 | -1.26 | 0.57 | -0.75 | 123.64 | 0.453 |
| Sleep | Week 2 | 66 | -3.22 | 0.17 | 61 | -3.01 | 0.17 | -0.20 | -0.68 | 0.27 | -0.85 | 415.15 | 0.399 |
| | Week 4 | 66 | -3.11 | 0.17 | 61 | -3.67 | 0.17 | 0.56 | 0.09 | 1.03 | 2.33 | 415.15 | 0.020 |
| | Week 6 | 66 | -3.50 | 0.17 | 61 | -3.93 | 0.17 | 0.43 | -0.04 | 0.90 | 1.78 | 415.15 | 0.076 |
| General feeling of sickness | Week 2 | 78 | -2.92 | 0.12 | 76 | -2.66 | 0.12 | -0.27 | -0.60 | 0.07 | -1.56 | 461.39 | 0.120 |
| | Week 4 | 78 | -3.35 | 0.12 | 76 | -3.42 | 0.12 | 0.07 | -0.26 | 0.41 | 0.43 | 461.39 | 0.665 |
| | Week 6 | 78 | -3.69 | 0.12 | 76 | -3.97 | 0.12 | 0.28 | -0.06 | 0.62 | 1.64 | 461.39 | 0.101 |

According to the SAP, only patients with severe complaints (baseline score of >3) were included in the analyses.

CI, confidence interval; LCL, lower confidence limit; n, number of patients with non-missing values used for the respective analysis; SE, standard error; UCL, upper confidence limit.

Two-sided t-test of contrasts within a mixed model for repeated measures; adjusted P values (see section 6).

Supplementary Table 12: Effects of nicotinamide on COVID-19 symptoms (change to baseline) in males of the RFITT population (complaint scale).

| Characteristics / symptoms | Time | Nicotinamide | | | Placebo | | | Contrast | 95% CI | | T | df | P |
|--------------------------------------|--------|--------------|-------|------|---------|-------|------|----------|--------|-------|-------|--------|-------|
| | | n | Mean | SE | n | Mean | SE | | LCL | UCL | | | |
| Ability to perform normal activities | Week 2 | 41 | -3.34 | 0.17 | 34 | -3.00 | 0.19 | -0.34 | -0.86 | 0.17 | -1.33 | 378.49 | 0.185 |
| | Week 4 | 41 | -3.66 | 0.17 | 34 | -3.53 | 0.19 | -0.13 | -0.64 | 0.38 | -0.51 | 378.49 | 0.611 |
| | Week 6 | 41 | -3.90 | 0.17 | 34 | -4.15 | 0.19 | 0.24 | -0.27 | 0.75 | 0.93 | 378.49 | 0.352 |
| Cough | Week 2 | 17 | -3.31 | 0.21 | 8 | -3.09 | 0.31 | -0.22 | -0.97 | 0.53 | -0.58 | 141.86 | 0.567 |
| | Week 4 | 17 | -3.54 | 0.21 | 8 | -3.72 | 0.31 | 0.17 | -0.58 | 0.92 | 0.46 | 141.86 | 0.649 |
| | Week 6 | 17 | -4.07 | 0.21 | 8 | -4.09 | 0.31 | 0.02 | -0.73 | 0.77 | 0.05 | 141.86 | 0.962 |
| Mucus production | Week 2 | 21 | -3.11 | 0.19 | 6 | -4.02 | 0.33 | 0.92 | 0.19 | 1.64 | 2.51 | 151.71 | 0.014 |
| | Week 4 | 21 | -3.92 | 0.19 | 6 | -3.69 | 0.33 | -0.23 | -0.95 | 0.49 | -0.63 | 151.71 | 0.533 |
| | Week 6 | 21 | -4.25 | 0.19 | 6 | -4.19 | 0.33 | -0.06 | -0.78 | 0.66 | -0.17 | 151.71 | 0.867 |
| Shortness of breath | Week 2 | 8 | -2.38 | 0.39 | 6 | -3.33 | 0.45 | 0.96 | -0.24 | 2.16 | 1.61 | 86.55 | 0.115 |
| | Week 4 | 8 | -2.88 | 0.39 | 6 | -3.00 | 0.45 | 0.12 | -1.08 | 1.33 | 0.21 | 86.55 | 0.835 |
| | Week 6 | 8 | -3.88 | 0.39 | 6 | -3.83 | 0.45 | -0.04 | -1.24 | 1.16 | -0.07 | 86.55 | 0.945 |
| Sleep | Week 2 | 32 | -3.34 | 0.21 | 25 | -3.49 | 0.23 | 0.15 | -0.46 | 0.77 | 0.49 | 358.54 | 0.622 |
| | Week 4 | 32 | -3.43 | 0.21 | 25 | -4.13 | 0.23 | 0.70 | 0.09 | 1.32 | 2.24 | 358.54 | 0.026 |
| | Week 6 | 32 | -3.96 | 0.21 | 25 | -4.17 | 0.23 | 0.21 | -0.41 | 0.82 | 0.67 | 358.54 | 0.503 |
| General feeling of sickness | Week 2 | 32 | -3.37 | 0.18 | 27 | -2.83 | 0.19 | -0.54 | -1.06 | -0.02 | -2.05 | 349.00 | 0.042 |
| | Week 4 | 32 | -3.68 | 0.18 | 27 | -3.57 | 0.19 | -0.11 | -0.63 | 0.41 | -0.42 | 349.00 | 0.674 |
| | Week 6 | 32 | -3.99 | 0.18 | 27 | -4.20 | 0.19 | 0.21 | -0.31 | 0.73 | 0.78 | 349.00 | 0.434 |

According to the SAP, only patients with severe complaints (baseline score of >3) were included in the analyses.

CI, confidence interval; LCL, lower confidence limit; n, number of patients with non-missing values used for the respective analysis; SE, standard error; UCL, upper confidence limit.

Two-sided, post-hoc, unadjusted t-test of contrasts within a mixed model for repeated measures.

Supplementary Table 13: Effects of nicotinamide on COVID-19 symptoms (change to baseline) in females of the RFITT population (complaint scale).

| Characteristics / symptoms | Time | Nicotinamide | | | Placebo | | | Contrast | 95% CI | | T | df | P |
|--------------------------------------|--------|--------------|-------|------|---------|-------|------|----------|--------|-------|-------|--------|-------|
| | | n | Mean | SE | n | Mean | SE | | LCL | UCL | | | |
| Ability to perform normal activities | Week 2 | 62 | -2.89 | 0.16 | 61 | -2.41 | 0.16 | -0.49 | -0.94 | -0.03 | -2.11 | 620.72 | 0.035 |
| | Week 4 | 62 | -3.38 | 0.16 | 61 | -3.24 | 0.16 | -0.13 | -0.59 | 0.32 | -0.58 | 620.72 | 0.561 |
| | Week 6 | 62 | -3.84 | 0.16 | 61 | -3.80 | 0.16 | -0.04 | -0.50 | 0.41 | -0.19 | 620.72 | 0.847 |
| Cough | Week 2 | 27 | -3.17 | 0.22 | 25 | -2.66 | 0.23 | -0.51 | -1.13 | 0.11 | -1.61 | 295.08 | 0.109 |
| | Week 4 | 27 | -3.35 | 0.22 | 25 | -3.34 | 0.23 | -0.01 | -0.63 | 0.61 | -0.04 | 295.08 | 0.968 |
| | Week 6 | 27 | -4.06 | 0.22 | 25 | -3.98 | 0.23 | -0.08 | -0.70 | 0.55 | -0.24 | 295.08 | 0.808 |
| Mucus production | Week 2 | 19 | -3.97 | 0.25 | 19 | -2.98 | 0.25 | -0.99 | -1.70 | -0.29 | -2.79 | 213.51 | 0.006 |
| | Week 4 | 19 | -3.44 | 0.25 | 19 | -3.61 | 0.25 | 0.16 | -0.54 | 0.87 | 0.46 | 213.51 | 0.648 |
| | Week 6 | 19 | -4.08 | 0.25 | 19 | -4.08 | 0.25 | 0.01 | -0.70 | 0.71 | 0.01 | 213.51 | 0.988 |
| Shortness of breath | Week 2 | 12 | -2.12 | 0.49 | 21 | -1.88 | 0.37 | -0.24 | -1.47 | 0.99 | -0.38 | 204.00 | 0.702 |
| | Week 4 | 12 | -2.70 | 0.49 | 21 | -2.50 | 0.37 | -0.20 | -1.43 | 1.03 | -0.33 | 204.00 | 0.745 |
| | Week 6 | 12 | -2.95 | 0.49 | 21 | -2.74 | 0.37 | -0.21 | -1.45 | 1.02 | -0.35 | 204.00 | 0.731 |
| Sleep | Week 2 | 34 | -3.10 | 0.25 | 36 | -2.69 | 0.24 | -0.41 | -1.09 | 0.28 | -1.17 | 440.31 | 0.244 |
| | Week 4 | 34 | -2.80 | 0.25 | 36 | -3.35 | 0.24 | 0.55 | -0.13 | 1.24 | 1.59 | 440.31 | 0.113 |
| | Week 6 | 34 | -3.07 | 0.25 | 36 | -3.77 | 0.24 | 0.71 | 0.02 | 1.39 | 2.03 | 440.31 | 0.044 |
| General feeling of sickness | Week 2 | 46 | -2.62 | 0.16 | 49 | -2.56 | 0.15 | -0.06 | -0.49 | 0.37 | -0.28 | 561.95 | 0.781 |
| | Week 4 | 46 | -3.12 | 0.16 | 49 | -3.34 | 0.15 | 0.21 | -0.22 | 0.65 | 0.97 | 561.95 | 0.331 |
| | Week 6 | 46 | -3.49 | 0.16 | 49 | -3.85 | 0.15 | 0.35 | -0.08 | 0.79 | 1.61 | 561.95 | 0.108 |

According to the SAP, only patients with severe complaints (baseline score of >3) were included in the analyses.

CI, confidence interval; LCL, lower confidence limit; n, number of patients with non-missing values used for the respective analysis; SE, standard error; UCL, upper confidence limit.

Two-sided, post-hoc, unadjusted t-test of contrasts within a mixed model for repeated measures.

Supplementary Table 14: Effects of nicotinamide on SF-36 scales (change to baseline) in the RFITT population.

| Subscale | Time | Nicotinamide | | | Placebo | | | Contrast | 95% CI | | T | df | P |
|----------------------------|--------|--------------|--------|------|---------|--------|------|----------|--------|-------|-------|--------|-------|
| | | n | Mean | SE | n | Mean | SE | | LCL | UCL | | | |
| Physical functioning | Week 2 | 113 | 31.62 | 2.02 | 111 | 32.89 | 2.04 | -1.28 | -6.93 | 4.38 | -0.44 | 385.08 | 0.657 |
| | Week 4 | 111 | 46.66 | 2.04 | 112 | 49.49 | 2.04 | -2.83 | -8.50 | 2.84 | -0.98 | 386.27 | 0.327 |
| | Week 6 | 110 | 55.08 | 2.04 | 111 | 57.98 | 2.04 | -2.91 | -8.59 | 2.77 | -1.01 | 388.81 | 0.315 |
| Role functioning/physical | Week 2 | 123 | 13.91 | 3.44 | 119 | 4.67 | 3.49 | 9.25 | -0.39 | 18.89 | 1.89 | 4.91 | 0.060 |
| | Week 4 | 121 | 32.41 | 3.46 | 120 | 27.87 | 3.48 | 4.53 | -5.12 | 14.19 | 0.92 | 4.91 | 0.357 |
| | Week 6 | 121 | 49.49 | 3.46 | 119 | 49.81 | 3.49 | -0.32 | -9.99 | 9.35 | -0.07 | 4.92 | 0.948 |
| Role functioning/emotional | Week 2 | 219 | -12.53 | 2.57 | 221 | -13.40 | 2.55 | 0.87 | -6.23 | 7.98 | 0.24 | 896.46 | 0.809 |
| | Week 4 | 216 | -1.87 | 2.58 | 223 | -4.45 | 2.55 | 2.58 | -4.53 | 9.69 | 0.71 | 897.43 | 0.477 |
| | Week 6 | 214 | 7.78 | 2.59 | 224 | 5.30 | 2.54 | 2.48 | -4.64 | 9.60 | 0.69 | 899.25 | 0.494 |
| Energy/fatigue | Week 2 | 122 | 2.70 | 1.76 | 113 | 1.30 | 1.83 | 1.41 | -3.59 | 6.41 | 0.55 | 369.11 | 0.580 |
| | Week 4 | 119 | 11.58 | 1.78 | 114 | 12.26 | 1.83 | -0.68 | -5.69 | 4.33 | -0.27 | 371.22 | 0.789 |
| | Week 6 | 118 | 20.70 | 1.78 | 114 | 21.68 | 1.83 | -0.98 | -6.00 | 4.04 | -0.38 | 372.31 | 0.701 |
| Emotional well-being | Week 2 | 132 | 1.05 | 1.34 | 121 | 0.23 | 1.40 | 0.82 | -3.00 | 4.64 | 0.42 | 399.92 | 0.672 |
| | Week 4 | 130 | 6.07 | 1.35 | 122 | 6.30 | 1.40 | -0.23 | -4.05 | 3.60 | -0.12 | 400.89 | 0.906 |
| | Week 6 | 127 | 12.56 | 1.36 | 122 | 12.22 | 1.40 | 0.34 | -3.50 | 4.17 | 0.17 | 404.16 | 0.864 |
| Social functioning | Week 2 | 116 | 3.50 | 2.17 | 115 | 3.98 | 2.19 | -0.48 | -6.54 | 5.58 | -0.16 | 470.98 | 0.876 |
| | Week 4 | 116 | 18.31 | 2.18 | 116 | 22.82 | 2.18 | -4.51 | -10.56 | 1.55 | -1.46 | 469.21 | 0.144 |
| | Week 6 | 115 | 34.84 | 2.18 | 116 | 34.78 | 2.18 | 0.06 | -6.00 | 6.13 | 0.02 | 470.74 | 0.984 |
| Pain | Week 2 | 125 | 9.48 | 1.97 | 120 | 5.16 | 2.02 | 4.32 | -1.23 | 9.87 | 1.53 | 479.79 | 0.126 |
| | Week 4 | 125 | 25.35 | 1.97 | 121 | 24.56 | 2.01 | 0.79 | -4.75 | 6.34 | 0.28 | 478.04 | 0.778 |
| | Week 6 | 125 | 38.85 | 1.97 | 121 | 38.34 | 2.01 | 0.51 | -5.03 | 6.06 | 0.18 | 478.04 | 0.856 |
| General health | Week 2 | 121 | 7.52 | 1.46 | 115 | 7.23 | 1.49 | 0.29 | -3.82 | 4.39 | 0.14 | 339.03 | 0.891 |
| | Week 4 | 118 | 12.77 | 1.47 | 116 | 12.02 | 1.49 | 0.75 | -3.36 | 4.86 | 0.36 | 341.04 | 0.719 |
| | Week 6 | 117 | 14.88 | 1.47 | 116 | 16.08 | 1.49 | -1.20 | -5.32 | 2.91 | -0.58 | 341.92 | 0.565 |
| Health change | Week 2 | 169 | 16.61 | 1.55 | 171 | 16.25 | 1.55 | 0.37 | -3.94 | 4.67 | 0.17 | 593.36 | 0.867 |
| | Week 4 | 166 | 24.35 | 1.56 | 170 | 24.80 | 1.55 | -0.45 | -4.77 | 3.87 | -0.21 | 598.57 | 0.837 |
| | Week 6 | 166 | 30.82 | 1.56 | 170 | 31.42 | 1.55 | -0.60 | -4.92 | 3.72 | -0.27 | 598.64 | 0.787 |

According to the SAP, only patients with severe complaints (baseline values \leq median) were included in the analyses.

CI, confidence interval; LCL, lower confidence limit; n, number of patients with non-missing values used for the respective analysis; SE, standard error; UCL, upper confidence limit.

Two-sided t-test of contrasts within a mixed model for repeated measures, adjusted for multiple timepoints.

Supplementary Table 15: Effects of nicotinamide on FACIT-F scales (change to baseline) in the RFITT population.

| Subscale / score | Time | Nicotinamide | | | Placebo | | | Contrast | 95% CI | | T | df | P |
|-----------------------------|--------|--------------|-------|------|---------|-------|------|----------|--------|------|-------|--------|-------|
| | | n | Mean | SE | n | Mean | SE | | LCL | UCL | | | |
| Physical well-being | Week 2 | 99 | 9.40 | 0.49 | 106 | 8.98 | 0.48 | 0.42 | -0.93 | 1.77 | 0.61 | 366.00 | 0.541 |
| | Week 4 | 99 | 12.96 | 0.49 | 106 | 12.95 | 0.48 | 0.00 | -1.35 | 1.35 | 0.00 | 365.15 | 0.998 |
| | Week 6 | 100 | 14.38 | 0.49 | 107 | 14.88 | 0.48 | -0.50 | -1.85 | 0.85 | -0.73 | 362.81 | 0.464 |
| Social/family well-being | Week 2 | 111 | 0.83 | 0.33 | 122 | 0.75 | 0.31 | 0.08 | -0.80 | 0.97 | 0.18 | 370.77 | 0.854 |
| | Week 4 | 109 | 1.28 | 0.33 | 122 | 1.35 | 0.31 | -0.07 | -0.95 | 0.83 | -0.14 | 373.38 | 0.887 |
| | Week 6 | 109 | 1.79 | 0.33 | 122 | 1.78 | 0.31 | 0.01 | -0.88 | 0.90 | 0.03 | 373.38 | 0.980 |
| Emotional well-being | Week 2 | 127 | 3.25 | 0.30 | 124 | 2.72 | 0.30 | 0.53 | -0.31 | 1.37 | 1.23 | 426.89 | 0.218 |
| | Week 4 | 123 | 4.50 | 0.30 | 125 | 4.19 | 0.30 | 0.31 | -0.54 | 1.15 | 0.72 | 430.73 | 0.475 |
| | Week 6 | 120 | 5.16 | 0.31 | 123 | 4.96 | 0.31 | 0.20 | -0.65 | 1.05 | 0.47 | 437.30 | 0.638 |
| Functional well-being | Week 2 | 117 | 5.77 | 0.54 | 123 | 5.61 | 0.52 | 0.16 | -1.31 | 1.63 | 0.22 | 400.96 | 0.828 |
| | Week 4 | 115 | 9.34 | 0.54 | 123 | 8.86 | 0.52 | 0.48 | -1.00 | 1.95 | 0.64 | 403.53 | 0.524 |
| | Week 6 | 114 | 11.30 | 0.54 | 124 | 11.34 | 0.52 | -0.04 | -1.52 | 1.43 | -0.06 | 403.71 | 0.955 |
| Fatigue | Week 2 | 112 | 16.09 | 1.05 | 112 | 15.97 | 1.06 | 0.12 | -2.82 | 3.05 | 0.08 | 382.10 | 0.937 |
| | Week 4 | 113 | 23.56 | 1.05 | 111 | 24.35 | 1.06 | -0.79 | -3.72 | 2.15 | -0.53 | 382.12 | 0.599 |
| | Week 6 | 112 | 27.65 | 1.05 | 112 | 27.86 | 1.06 | -0.21 | -3.14 | 2.73 | -0.14 | 381.95 | 0.891 |
| FACIT-F Trial Outcome Index | Week 2 | 97 | 27.91 | 2.03 | 99 | 29.51 | 2.01 | -1.60 | -7.22 | 4.02 | -0.56 | 334.14 | 0.576 |
| | Week 4 | 98 | 42.37 | 2.02 | 98 | 45.45 | 2.02 | -3.08 | -8.70 | 2.53 | -1.08 | 334.02 | 0.281 |
| | Week 6 | 97 | 50.88 | 2.03 | 100 | 52.99 | 2.01 | -2.11 | -7.72 | 3.50 | -0.74 | 332.71 | 0.460 |
| FACT-G Score | Week 2 | 91 | 15.87 | 1.50 | 101 | 15.25 | 1.43 | 0.61 | -3.47 | 4.70 | 0.30 | 304.45 | 0.768 |
| | Week 4 | 90 | 24.00 | 1.51 | 100 | 25.30 | 1.43 | -1.30 | -5.40 | 2.80 | -0.62 | 306.74 | 0.533 |
| | Week 6 | 87 | 29.30 | 1.52 | 102 | 29.68 | 1.43 | -0.38 | -4.49 | 3.72 | -0.18 | 308.88 | 0.854 |
| FACIT-F General Score | Week 2 | 94 | 31.23 | 2.47 | 94 | 32.34 | 2.48 | -1.11 | -8.01 | 5.79 | -0.32 | 309.36 | 0.752 |
| | Week 4 | 94 | 47.24 | 2.47 | 91 | 50.16 | 2.50 | -2.91 | -9.84 | 4.02 | -0.83 | 313.16 | 0.409 |
| | Week 6 | 92 | 56.47 | 2.48 | 94 | 57.71 | 2.48 | -1.23 | -8.16 | 5.69 | -0.35 | 311.88 | 0.726 |

According to the SAP, only patients with severe complaints (baseline values \leq median) were included in the analyses.

CI, confidence interval; LCL, lower confidence limit; n, number of patients with non-missing values used for the respective analysis; SE, standard error; UCL, upper confidence limit.

Two-sided t-test of contrasts within a mixed model for repeated measures, adjusted for multiple timepoints.

2.3. Supplementary clinical data (text)

Levels of antibodies directed against the nucleocapsid (N) or spike (S) proteins of SARS-CoV-2 after at least 6 months (secondary endpoint)

For the RFITT population, no differences between the trial arms were observed with respect to the anti-N antibodies ($D = 2.7$, $p = 0.493$) and anti-S antibodies ($D = 3.0$, $p = 0.983$). Within the subgroup of vaccinated patients, no significant difference (nicotinamide vs. placebo) was seen for anti-N antibodies ($D = 0.10$; $p = 0.985$) and anti-S antibodies ($D = -6.2$, $p = 0.974$). For the subgroups of unvaccinated patients, differences between the nicotinamide and placebo groups with respect to anti-N antibodies ($D = 7.8$, $p = 0.248$) and anti-S antibodies ($D = 46.0$, $p = 0.137$) were also not significant. With respect to anti-S antibodies, RFITT responders to the nicotinamide intervention showed a non-significant difference (nicotinamide vs. placebo) of $D = 154.0$, $p = 0.363$, in the subgroup of vaccinated responders ($D = 138.0$, $p = 0.530$) and in the subgroup of unvaccinated responders ($D = 55.0$, $p = 0.135$).

Symptom resolution over time (secondary endpoints)

In the interviews at weeks 2, 4 and 6, patients were asked whether they were free of individual or all symptoms. Using this variable, 60 patients (24.19%) of the for RFITT population receiving nicotinamide and 48 patients (19.05%) receiving placebo reported to be symptom-free at week 2 ($p = 0.192$). At week 4, 103 patients (41.53%) receiving nicotinamide and 101 patients (40.08%) receiving placebo reported to be symptom-free ($p = 0.785$). At week 6, 129 patients (52.02%) receiving nicotinamide and 129 patients (51.19%) receiving placebo reported to be symptom-free ($p = 0.858$). For primary and key secondary endpoints as well as for being completely symptom-free, the time to resolution was analyzed using a Kaplan-Meier analysis. Only symptomatic patients who had the respectively symptom at baseline (binary queries) or showed symptom scores greater than 3 (ordinal queries) (see section 3.2 of this Supplement) were included. ‘Symptom-free’ was defined as a rating of zero on a scale of complaints. For RFITT patients, no significant differences between the trial arms were observed for time to resolution of performance drop (HR 0.83; 95% CI [0.66; 1.03]; $p = 0.087$), the ability to perform normal activities (HR 0.72; 95% CI [0.52; 1.00]; $p = 0.051$), fatigue (HR 0.92; 95% CI [0.75; 1.14]; $p = 0.453$) cough (HR 0.81; 95% CI [0.49; 1.34]; $p = 0.414$) or being completely symptom-free (HR 0.91; 95% CI [0.73; 1.13]; $p = 0.79$). The time from study inclusion (screening interview) to resolution of individual symptoms (in days) as reported within the patients’ interviews was analyzed using Cox regression. For RFITT, no difference between the interventions was observed (HR 0.89; 95% CI [0.70; 1.13]; $p = 0.327$).

WHO COVID-19 ordinal scale for clinical improvement and occurrence of severe COVID-19 (exploratory endpoints)

The results are summarized in the safety evaluation in section 2.5 of this Supplement (Supplementary Tables 25–29).

Molecular exploratory endpoints

For the exploratory endpoints blood biomarkers, blood metabolome, SARS-CoV-2 strains and pharmacokinetics., quarantine rules did not allow us to acquire biosamples from more than 12 local patients. Therefore, a formal analysis would lack statistical power, but data can be obtained from the corresponding author.

2.4. Supplementary data from gut microbiome analyses

To assess longitudinal shifts in fecal microbial communities associated with nicotinamide, we performed 16S rDNA phylogenomic profiling and metagenomic sequencing analyses. DNA of stool samples of 88 independent patients (70 patients for 16S and 18 patients for metagenomics), which were collected at week 0 (baseline), weeks 2 and 4 (exposure to nicotinamide or placebo) and week 6 (follow-up), was extracted and subjected to 16S rRNA (n=280) and shotgun metagenomics (n=72) sequencing. A schematic study design is depicted in Fig. 2a.

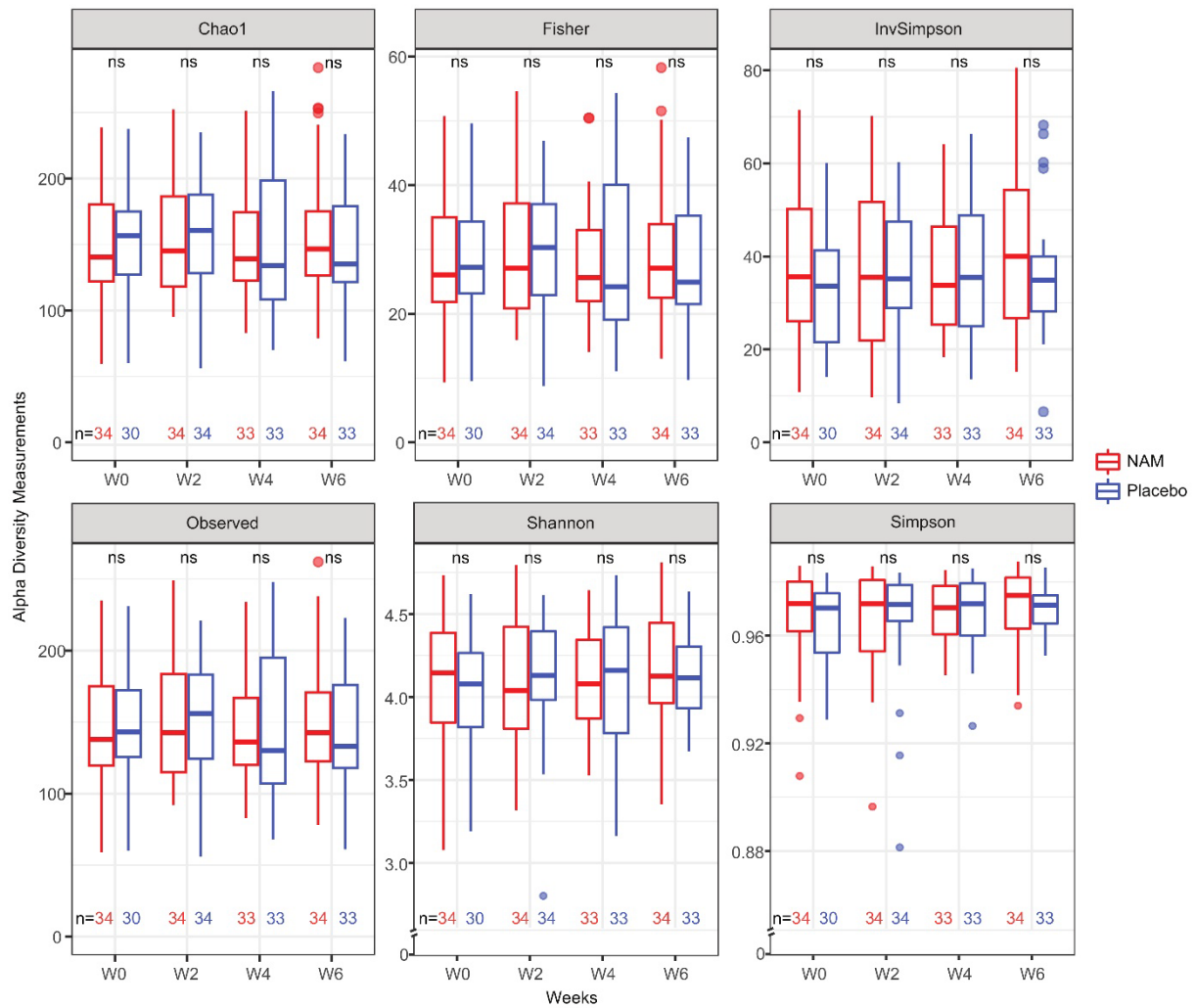
After quality control, different α -diversity (within sample diversity) analyses of 16S rDNA data did not show significant longitudinal or cross-sectional differences between intervention groups (Fig. 2b and Supplementary Fig. 3).

β -diversity (between sample diversity) analyses (Aitchison distance) did not reveal a significant difference between intervention groups at baseline (Supplementary Table 19). Longitudinal, intervention-dependent β -diversity differences were computed using global scaling and constraint analyses of principal coordinates (CAP) on Aitchison distances from baseline. Differences between nicotinamide and placebo during the intervention period (week 2 and week 4) were tested with PERMANOVA with 10,000 permutations (Fig. 2c; Supplementary Table 18). Here, the gut microbiome composition of patients receiving nicotinamide significantly differed from those receiving placebo ($R^2 = 0.015$, false discovery rate [FDR] = 0.002). Interestingly, when identifying contributive factors associated with dissimilarity, we demonstrate that key COVID-19-related symptoms (fever, cough, impaired sense of smell or impaired sense of taste at baseline) ($R^2 = 0.0067$, FDR = 0.013) and placebo had a similar effect in the post-baseline samples, which was opposite to the nicotinamide group (Fig. 2c, Supplementary Table 18). Likewise, we ran a principal coordinate analysis of the 16S rRNA amplicon sequences (Supplementary Figs 4a and 4b) and metagenomics (Supplementary Figs 4c and 4d), suggesting similar microbial compositions among both cohorts.

A formal variance partition analysis was conducted at baseline to evaluate the contribution of patient covariates to the genus-level microbial composition. Among the factors, fever before baseline showed the highest contribution to the variance (3%), followed by body mass index (BMI) (2.7%), age (2.7%), and other COVID-19-specific symptoms (1.7%) (Supplementary Fig. 5, Supplementary Table 24). The highest variance contribution by fever confirms that the microbial composition of patients with COVID-19 seems to be driven by disease severity, which has been suggested previously.¹ When stratifying α -diversity indices for the presence of fever at baseline in patients with key COVID-19-related symptoms using mixed-effect regressions, we found that also within sample diversity was decreased in affected patients at early timepoints, with varying effect sizes for different indices (Supplementary Fig. 6). Significantly sustained reduction of α -diversity in patients with fever at baseline in the placebo arm was observed in Shannon and Simpson indices. No significant α -diversity reduction (Shannon and Simpson) was observed in patients receiving nicotinamide at timepoints week 4 and 6.

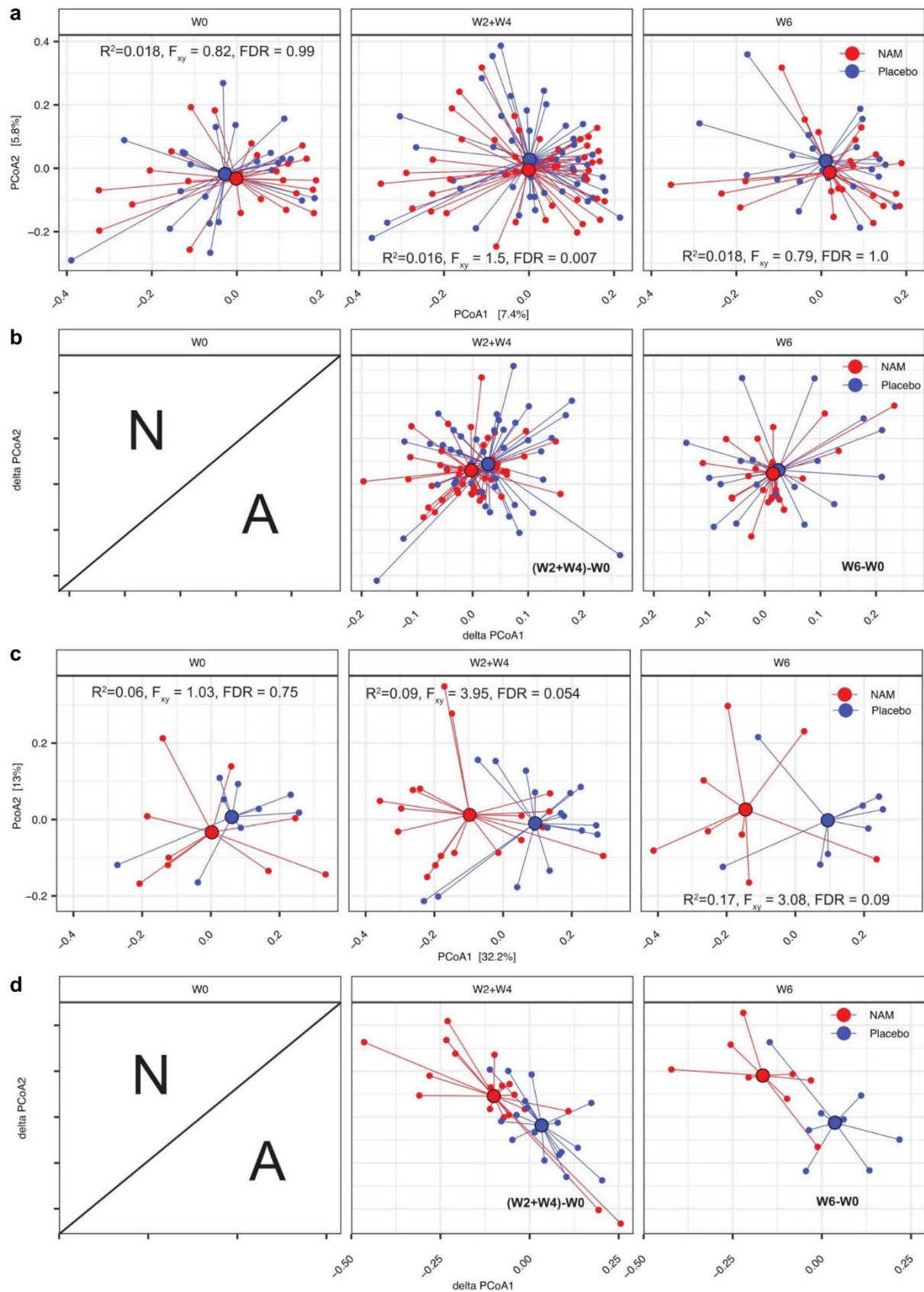
Further variance partition analyses were done to identify the top 20 most variable genera associated to nicotinamide intervention in the 16S rDNA amplicon sequencing cohort (Fig. 2d, Supplementary Table 20) and in the metagenomics cohort (Supplementary Fig. 7a, Supplementary Table 21) at week 2 and week 4 of intervention. We found an overlap in 11 genera from the top 50 most variable genera between both cohorts (Supplementary Fig. 7b).

To address the putative metabolic potential of fecal microbial communities according to the respective intervention groups, we analyzed metagenomic data from an independent trial patient set. Using assignment of taxonomic features and quantification of microbial communities by MetaPhlAn 3.0 and inference of functional potential profiling by HUMAnN 3.0,^{ref. 2} we observed high abundance of amino acid, cofactor and vitamin biosynthesis pathways. Strikingly, there was a significant increase of tryptophan biosynthesis in patients receiving placebo compared to patients receiving nicotinamide at week 2, indicating a higher demand for *de novo* synthesis of this amino acid as expected from earlier findings. This shift was reduced in samples from the nicotinamide group. Note that at the functional level the strongest difference between placebo and active arm was observed at week 2, which reflects the timepoint with the strongest clinical effect of nicotinamide (Fig. 3). A complete list of abundance shifts of inferred pathways according to intervention and timepoint is presented in Supplementary Table 22. Overall, the findings indicate an influence of symptomatic COVID-19 on gut microbial communities, which was influenced by nicotinamide as a metabolic intervention.



Supplementary Fig. 3: α -diversity metrics in relation to the intervention.

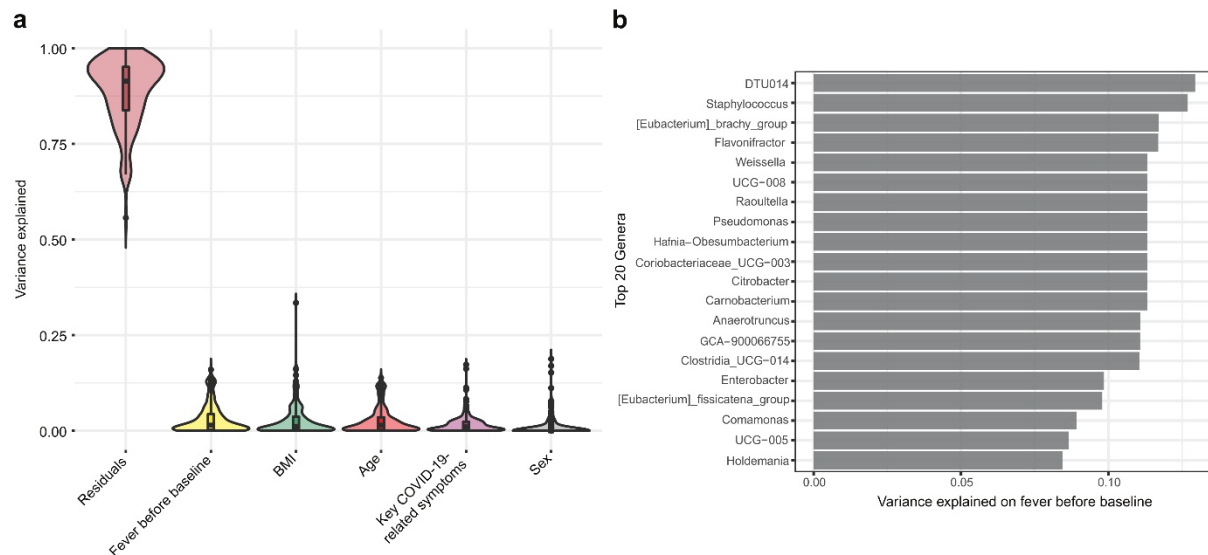
All the indexes were computed at the Amplicon Sequence Variant (ASV) level. Cross-sectional comparisons between nicotinamide (NAM) and placebo were performed using the two-sided Wilcoxon rank-sum test (n per group is depicted below each box plot; ns, not significant). Only samples with a minimum of 5,000 reads were included. Linear mixed-effect models with random intercepts for subjects evaluated the effect of intervention time. A likelihood ratio test comparing the full and null models showed no significant effect of time. Box plots show the median (center line), interquartile range (IQR, box), 1.5x IQR (whiskers) and outliers (points).



Supplementary Fig. 4 | Principal Coordinate Analysis (PCoA) based on Bray-Curtis distances showing microbial community composition changes in patients with key COVID-19-related symptoms.

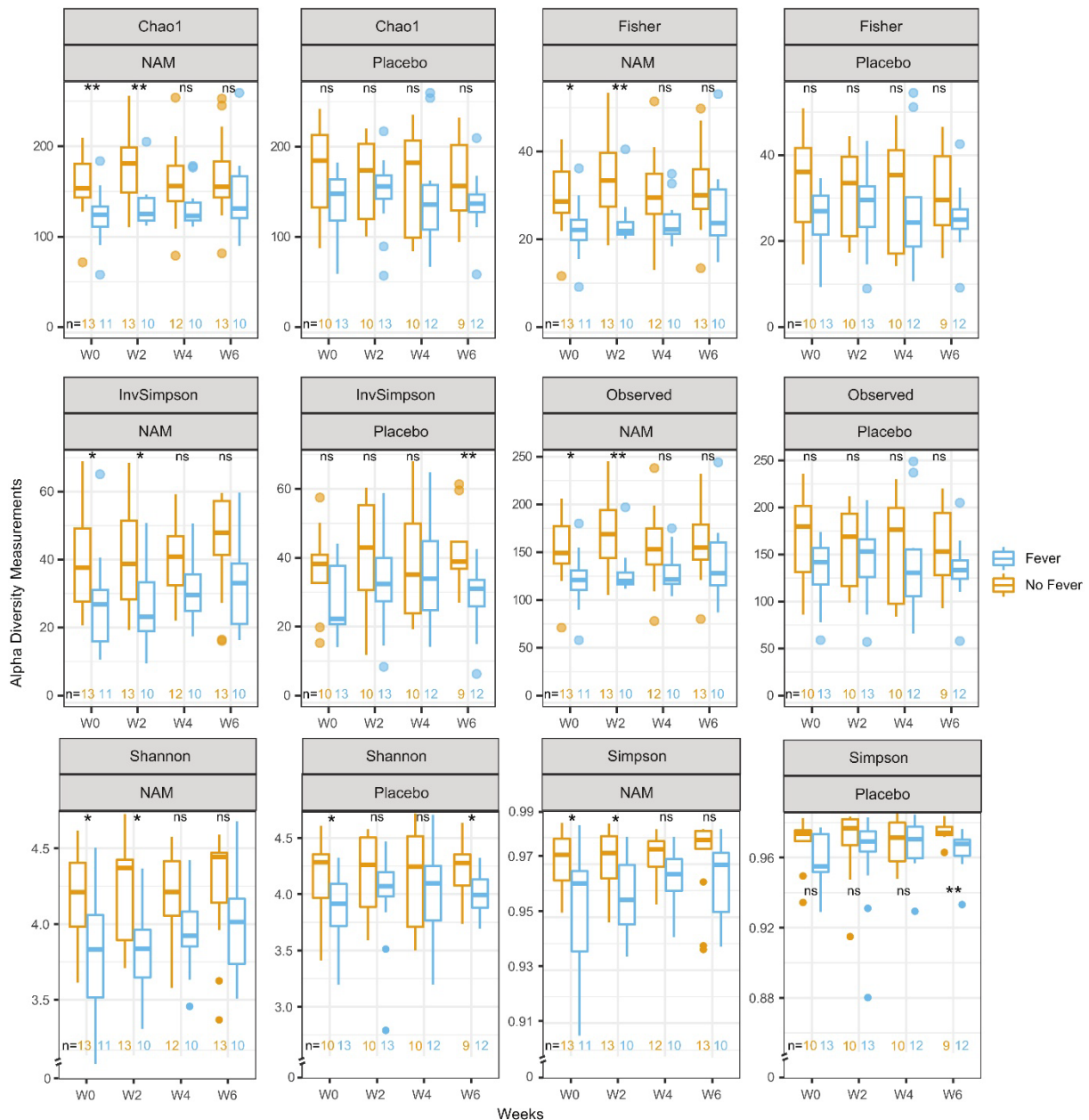
a, Cohort 1, 16S rRNA gene sequencing data. PCoA plots show microbial community composition at baseline (week 0 [W0]; 24 patients receiving nicotinamide [NAM], 23 placebo), weeks 2–4 (W2+W4; n=45 per intervention), and week 6 (W6; n=23 NAM, n=21 placebo). Significant differences between the NAM and placebo

were observed at W2+W4 ($R^2=0.016$, $F=1.50$, false discovery rate [FDR]=0.007). Individual samples are connected to the group centroids for each timepoint. **b**, Cohort 1, 16S rRNA gene sequencing data (W0: n=24 NAM, n=23 placebo; W2+W4: n=45 per intervention; W6: n=23 NAM, n=21 placebo). Delta PCoA plots representing changes in principal coordinates relative to baseline (W0). For each sample, the baseline principal coordinates were subtracted from the corresponding intervention timepoints ((W2+W4) – W0) or follow-up timepoints (W6 – W0). This delta visualization emphasizes shifts in community composition during the intervention (W2+W4) and follow-up (W6) phases and was calculated as described by Maifeld et al. (2021).³ N/A, not applicable. **c**, Cohort 2, shotgun metagenomics data (n=9 per intervention and timepoint). PCoA plots at the same time points as in panel **a** show a trend toward significance at W2+W4 ($R^2=0.09$, $F=3.95$, FDR=0.054) and W6 ($R^2=0.17$, $F=3.08$, FDR=0.09). **d**, Cohort 2, shotgun metagenomics data (n=9 per intervention and timepoint). Delta PCoA plots analogous to panel **b**, showing changes in principal coordinates relative to baseline (W0). The delta approach captures temporal shifts in microbial community composition for NAM and placebo groups during intervention and follow-up phases. PERMANOVA test with 10,000 permutations for intervention effects were done on the original distance matrices using the *adonis* function in the *vegan* (2.5-5) R package, stratified for patient ID (see section 3.5 of this Supplement). Key COVID-19-related symptoms were fever, cough, impaired sense of smell or impaired sense of taste at baseline. Only samples with a minimum of 5,000 reads were included. FDR represents Benjamini-Hochberg-corrected P values. N/A, not applicable.



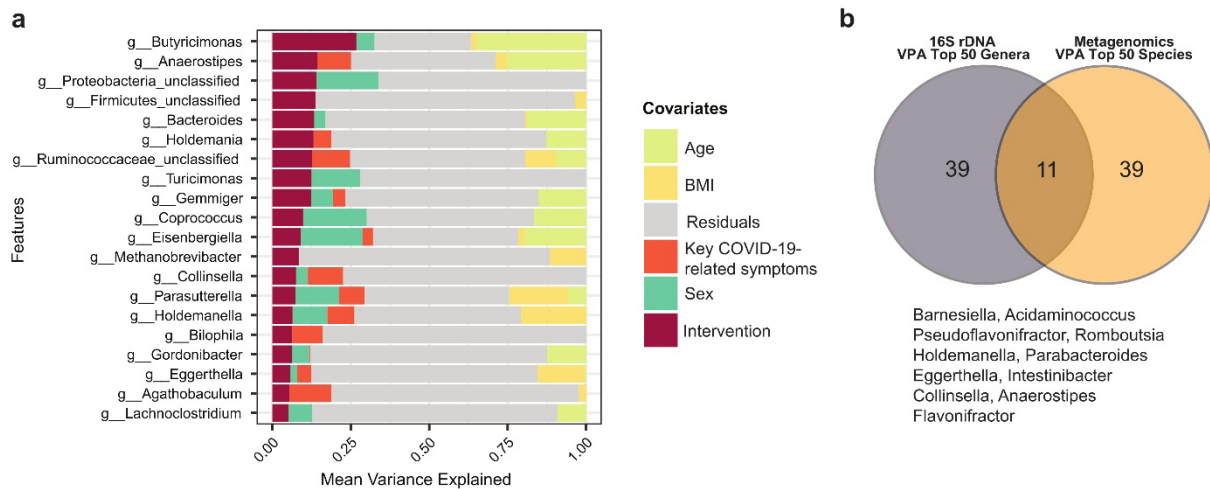
Supplementary Fig. 5: Variance contribution of baseline characteristics.

a, The effects of important patient covariates on the genus-level gut microbiome composition at baseline were evaluated using a variance partition analysis (34 patients receiving nicotinamide, 30 placebo; see also Supplementary Table 24). Only samples with a minimum of 5,000 reads were included. Fever before baseline showed the highest contribution to the variance (3%), followed by body mass index (BMI) (2.7%), age (2.7%), and other key COVID-19-related symptoms (1.7%). **b**, The 20 most important genera contributing to the variance associated with fever before baseline.



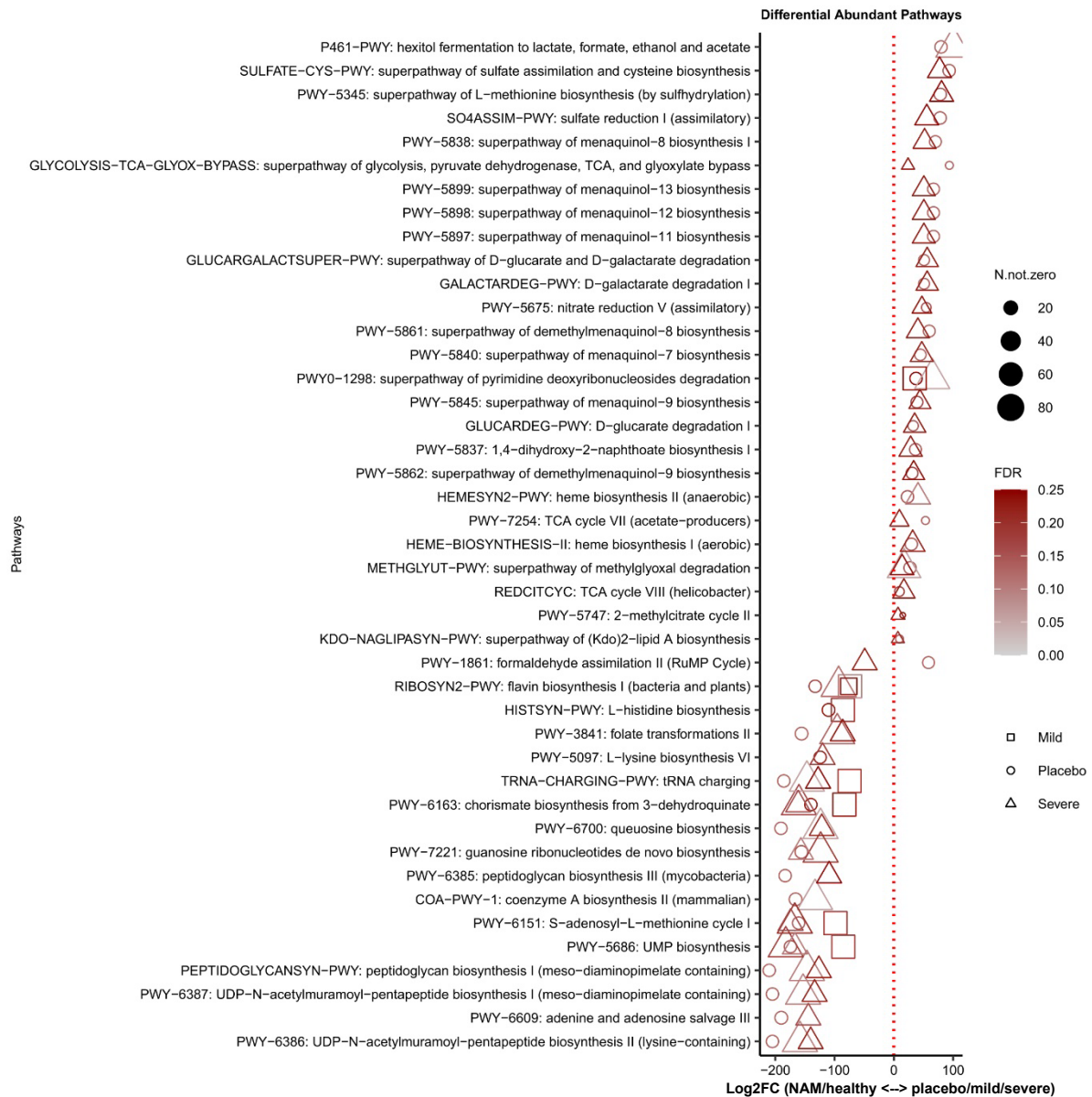
Supplementary Fig. 6: Longitudinal analysis of α -diversity in relation to fever in patients with key COVID-19-related symptoms.

Shifts in microbial diversity within samples were analyzed by α -diversity measurements. Among the genera mostly contributing to those differences we found *Staphylococcus*, *Pseudomonas*, and *Citrobacter*. All indices were computed at the Amplicon Sequence Variant (ASV) level. Cross-sectional comparisons between ‘Fever’ and ‘No Fever’ at baseline were performed using the two-sided Wilcoxon rank-sum test (n per group is depicted below each box plot). Asterisks represent significant P values (* P<0.05, ** P<0.01), corrected for multiple comparisons. Box plots show the median (center line), interquartile range (IQR, box), 1.5x IQR (whiskers) and outliers (points). Key COVID-19-related symptoms were fever, cough, impaired sense of smell or impaired sense of taste at baseline. Only samples with a minimum of 5,000 reads were included. NAM, nicotinamide.



Supplementary Fig. 7 | Variance Partition Analysis (VPA) of microbial genera and comparison of 50 top genera identified in 16S rRNA gene sequencing and shotgun metagenomics.

a, VPA of the top 20 microbial genera that present most variation from the nicotinamide intervention in the shotgun metagenomics data ($n=9$ per intervention). The bar plot shows the mean variance explained for the top 20 microbial genera, with variance attributed to covariates including age (light green), body mass index (BMI; yellow), residuals (gray), COVID-19 symptoms (red), sex (dark green), and intervention (purple). Features are displayed at the genus level. **b**, Comparison of top 50 genera between 16S rRNA gene sequencing and shotgun metagenomics. The Venn diagram illustrates the overlap of the top 50 genera identified by VPA in 16S sequencing (dark gray circle) and shotgun metagenomics (orange circle). 11 genera were shared between the two technologies, including *Barnesiella*, *Acidaminococcus*, *Pseudoflavonifractor*, *Romboutsia*, *Holdemanella*, *Parabacteroides*, *Eggerthella*, *Intestinibacter*, *Collinsella*, *Anaerostipes* and *Flavonifractor*.



Supplementary Fig. 8 | 43 functional pathways overlap in the gut microbiota of healthy control individuals vs. patients with mild or severe COVID-19 in the public dataset from Essex et al. and nicotinamide (NAM)-receiving vs. placebo-receiving participants in COVIt-2.

The dot plot represents the effect size of the differential abundant pathways between the NAM/healthy and placebo/mild/severe groups. Healthy (n=15), mild (n=15), and severe (n=8) groups were from Essex et al.¹³ and NAM (n=9) and placebo (n=9) groups were from the COVIt-2 trial. The x-axis represents log2 fold change (Log2FC), with negative values indicating enrichment in NAM/healthy groups and positive values indicating enrichment in placebo/mild/severe groups. Symbol size reflects the number of samples in which the pathway was detected (N.not.zero), and the false discovery rate (FDR) color scale represents the Benjamini-Hochberg-corrected P values gradient.

Supplementary Table 16: Demographic and clinical characteristics at baseline of the 16S phylogenomic subcohort (n=70).

| | Levels | Nicotinamide | Placebo | Total | P |
|---|-----------|--------------|-------------|-------------|-------|
| Total N (%) | | 35 (50.0) | 35 (50.0) | 70 (100.0) | |
| Age | Mean (SD) | 41.2 (11.4) | 42.4 (10.7) | 41.8 (11.0) | 0.66 |
| Sex | Female | 25 (71.4) | 24 (68.6) | 49 (70.0) | 1 |
| | Male | 10 (28.6) | 11 (31.4) | 21 (30.0) | |
| Race | White | 35 (100.0) | 33 (94.3) | 68 (97.1) | 0.493 |
| | Other | 0 (0.0) | 2 (5.7) | 2 (2.9) | |
| Body mass index (BMI) | Mean (SD) | 23.9 (5.9) | 25.4 (5.3) | 24.6 (5.6) | 0.26 |
| Age > 60 yr | Yes | 2 (5.7) | 1 (2.9) | 3 (4.3) | 1 |
| | No | 33 (94.3) | 34 (97.1) | 67 (95.7) | |
| BMI >30 and/or type 2 diabetes | Yes | 5 (14.3) | 7 (20.0) | 12 (17.1) | 0.752 |
| | No | 30 (85.7) | 28 (80.0) | 58 (82.9) | |
| Cardiovascular diseases, high blood pressure or stroke | Yes | 3 (8.6) | 3 (8.6) | 6 (8.6) | 1 |
| | No | 32 (91.4) | 32 (91.4) | 64 (91.4) | |
| Asthma, chronic obstructive pulmonary disease, or other chronic lung diseases | Yes | 0 (0.0) | 4 (11.4) | 4 (5.7) | 0.114 |
| | No | 35 (100.0) | 31 (88.6) | 66 (94.3) | |

Fisher's exact test was used to compare variables between nicotinamide and placebo groups. No significant differences were found.

Supplementary Table 17: Demographic and clinical characteristics of the metagenomics subcohort (n=18).

| | Levels | Nicotinamide | Placebo | Total | P |
|---|-----------|--------------|-------------|-------------|-------|
| Total N (%) | | 9 (50.0) | 9 (50.0) | 18 | |
| Age | Mean (SD) | 37.7 (12.8) | 38.6 (10.1) | 38.1 (11.2) | 0.872 |
| Sex | Female | 4 (44.4) | 5 (55.6) | 9 (50.0) | 1 |
| | Male | 5 (55.6) | 4 (44.4) | 9 (50.0) | |
| Race | White | 9 (100.0) | 9 (100.0) | 18 (100.0) | 1 |
| | Other | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Body mass index (BMI) | Mean (SD) | 28.0 (8.2) | 27.1 (7.0) | 27.6 (7.4) | 0.808 |
| Age > 60 yr | Yes | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 |
| | No | 9 (100.0) | 9 (100.0) | 18 (100.0) | |
| BMI >30 and/or type 2 diabetes | Yes | 2 (22.2) | 3 (33.3) | 5 (27.8) | 1 |
| | No | 7 (77.8) | 6 (66.7) | 13 (72.2) | |
| Cardiovascular diseases, high blood pressure or stroke | Yes | 4 (44.4) | 1 (11.1) | 5 (27.8) | 0.294 |
| | No | 5 (55.6) | 8 (88.9) | 13 (72.2) | |
| Asthma, chronic obstructive pulmonary disease, or other chronic lung diseases | Yes | 2 (22.2) | 2 (22.2) | 4 (22.2) | 1 |
| | No | 7 (77.8) | 7 (77.8) | 14 (77.8) | |

Fisher's exact test was used to compare variables between nicotinamide and placebo groups. No significant differences were found.

Supplementary Table 18: PERMANOVA results of the influence of age, key COVID-19-related symptoms and intervention on the gut microbial composition of the 16S phylogenomic subcohort (n=70) at week 2 and week 4.

| Covariate | Df | SumOfSqs | R ² | F | P | parOmegaSq | FDR |
|-------------------------------|-----|-----------|----------------|------|--------|------------|--------|
| Age | 1 | 5730.84 | 0.019 | 3.43 | 0.0001 | 0.0133 | 0.0005 |
| Key COVID-19-related symptoms | 1 | 2065.91 | 0.007 | 1.24 | 0.0095 | 0.0013 | 0.0158 |
| Intervention | 2 | 4784.09 | 0.016 | 1.43 | 0.0012 | 0.0048 | 0.002 |
| Residual | 176 | 293762.09 | 0.959 | NA | NA | NA | NA |
| Total | 180 | 306342.93 | 1.000 | NA | NA | NA | NA |

PERMANOVA was tested with 10000 permutations.

Df, degrees of freedom; F, pseudo-F statistics value; FDR, false discovery rate; P, P value; parOmegaSq, Partial Omega Squared value; R², variation explained; SumOfSqs, sum of squares.

Supplementary Table 19: PERMANOVA results of the influence of age and intervention on the gut microbial composition of the 16S phylogenomic subcohort (n=70) at baseline.

| Covariate | Df | SumOfSqs | R ² | F | P | parOmegaSq | FDR |
|--------------|----|----------|----------------|------|--------|------------|--------|
| Age | 1 | 1760.5 | 0.024 | 1.08 | 0.1836 | 0.0018 | 0.7343 |
| Intervention | 1 | 1421.88 | 0.019 | 0.88 | 0.9187 | 0.0027 | 1 |
| Residual | 44 | 71484.3 | 0.957 | NA | NA | NA | NA |
| Total | 46 | 74666.67 | 1 | NA | NA | NA | NA |

PERMANOVA was tested with 10000 permutations.

Df, degrees of freedom; F, pseudo-F statistics value; FDR, false discovery rate; P, P value; R², variation explained; SumOfSqs, sum of squares.

Supplementary Table 20: Proportion of variation explained by each feature and confounder in the 16S phylogenomic subcohort (n=70) at weeks 2 and 4, based on 16S data.

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|---|-------------|-------------|-----------------------|-------------|-------------|-------------------------------|--------------|
| Genus63 Anaerotruncus | 0.769987225 | 0 | 0.109467138 | 0 | 1.75E-10 | 0 | 0.120545637 |
| Genus188 Frisingicoccus | 0.674086754 | 0.127329328 | 0.085529187 | 2.36E-11 | 0 | 0 | 0.11305473 |
| Genus71 unknown | 0.78692853 | 0.058559064 | 0.024222356 | 0.031140058 | 0 | 0 | 0.099149992 |
| Genus7 Barnesiella | 0.749122481 | 0.11717493 | 0.018333896 | 0 | 1.38E-13 | 0.024585022 | 0.090783671 |
| Genus124 Adlercreutzia | 0.653746004 | 0.054534943 | 0.012670459 | 0.046641635 | 0.146767381 | 0 | 0.085639578 |
| Genus113 Bifidobacterium | 0.779860892 | 0.11790051 | 0.020807541 | 0 | 1.01E-12 | 0.000731139 | 0.080699918 |
| Genus209 Hungatella | 0.653099295 | 0.060249946 | 0.164876085 | 0.010492298 | 0.030591396 | 1.09E-12 | 0.08069098 |
| Genus162 Acidaminococcus | 0.772568702 | 0.041298399 | 4.76E-11 | 0.052117539 | 0.015927837 | 0.038130396 | 0.079957127 |
| Genus197 [Eubacterium]_ruminantium_group | 0.84463795 | 3.14E-13 | 0.035954949 | 0 | 0.029526954 | 0.022827772 | 0.067052376 |
| Genus105 unknown | 0.89626879 | 0.016918082 | 0.026002396 | 2.23E-11 | 6.20E-12 | 3.69E-10 | 0.060810733 |
| Genus60 unknown | 0.791738611 | 0.115059016 | 9.03E-12 | 0.005272826 | 0 | 0.028570504 | 0.059359043 |
| Genus196 [Eubacterium]_xylanophilum_group | 0.560067609 | 0.156603881 | 0 | 0.04604547 | 0.152227508 | 0.028264884 | 0.056790648 |
| Genus72 Pseudoflavonifractor | 0.911050832 | 0.003171152 | 3.53E-12 | 0 | 0.031964057 | 0 | 0.05381396 |
| Genus176 [Eubacterium]_nodatum_group | 0.764132646 | 0.097360039 | 0.090964878 | 4.88E-12 | 0 | 0 | 0.047542437 |
| Genus202 Howardella | 0.826282588 | 0.032518382 | 4.02E-13 | 0.074133139 | 0.019561971 | 0 | 0.047503919 |
| Genus192 Lactococcus | 0.920355606 | 0.010793078 | 3.75E-11 | 0 | 0.011930903 | 0.010811888 | 0.046108525 |
| Genus48 Incertae_Sedis | 0.783072447 | 0 | 0.053499216 | 0.103229407 | 0 | 0.015828092 | 0.044370839 |
| Genus190 GCA-900066575 | 0.914233017 | 0 | 0 | 0.013561535 | 0 | 0.028947507 | 0.043257941 |
| Genus98 Oxalobacter | 0.906578667 | 0.027607127 | 0.025161463 | 0 | 0 | 0 | 0.040652743 |
| Genus131 RF39 | 0.884823999 | 0.033860289 | 0.011509944 | 0 | 0.029907778 | 1.26E-11 | 0.03989799 |
| Genus177 Romboutsia | 0.761557654 | 0.172920928 | 0.02937148 | 0 | 0 | 0 | 0.036149938 |
| Genus215 Lachnospiraceae_UCG-004 | 0.874363202 | 0.088576773 | 4.22E-10 | 0 | 0.000941472 | 0 | 0.036118553 |
| Genus29 [Ruminococcus]_torques_group | 0.683759724 | 0.049102049 | 0.054919256 | 0.178545507 | 0 | 0.00011627 | 0.033557194 |
| Genus150 Holdemanella | 0.95698056 | 0 | 1.77E-05 | 0.013086467 | 0 | 0 | 0.029915248 |
| Genus54 Faecalibacterium | 0.896775553 | 0.058872273 | 2.84E-11 | 0 | 0 | 0.015614413 | 0.028737761 |

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|--|-------------|-------------|-----------------------|-------------|-------------|-------------------------------|--------------|
| Genus55 Subdoligranulum | 0.925586059 | 0.019203555 | 0.025258035 | 0 | 0 | 0.001254372 | 0.02869798 |
| Genus111 Victivallaceae | 0.938100151 | 0.034084722 | 3.56E-11 | 1.04E-11 | 0 | 0 | 0.027815127 |
| Genus44 Clostridia_UCG-014 | 0.552718893 | 0.133444753 | 0.116428434 | 0.068319897 | 0.101385275 | 0 | 0.027702749 |
| Genus24 Parabacteroides | 0.921353499 | 0.045388949 | 0 | 0.005662525 | 0 | 0 | 0.027595028 |
| Genus53 Fournierella | 0.783563092 | 0.106201622 | 1.18E-11 | 0 | 0.014332756 | 0.075515051 | 0.020387479 |
| Genus89 Proteus | 0.980840324 | 5.23E-18 | 0 | 0 | 0 | 0 | 0.019159676 |
| Genus5 unknown | 0.879701222 | 0.0263693 | 0.042708164 | 0 | 0 | 0.032069345 | 0.01915197 |
| Genus1 Alistipes | 0.752955646 | 0 | 0.02749562 | 0.185572908 | 0 | 0.01645824 | 0.017517586 |
| Genus67 Butyrivibrio | 0.832089195 | 0.046539235 | 3.05E-13 | 0 | 0.043205353 | 0.062438813 | 0.015727404 |
| Genus74 Colidextribacter | 0.951283845 | 0.033258188 | 1.04E-11 | 0 | 0 | 0 | 0.015457967 |
| Genus122 Eggerthella | 0.694730878 | 0.181827377 | 0.108272744 | 0 | 1.87E-13 | 0 | 0.015169001 |
| Genus179 Intestinibacter | 0.960621759 | 0 | 0.024875623 | 2.32E-13 | 0 | 0 | 0.014502618 |
| Genus129 Collinsella | 0.792722507 | 0.150221576 | 0.039676419 | 0 | 0 | 0.003920782 | 0.013458716 |
| Genus112 Actinomyces | 0.88829435 | 0 | 0.071061856 | 0.024181708 | 0 | 0.003298184 | 0.013163903 |
| Genus49 DTU089 | 0.929371192 | 0.051892611 | 1.06E-10 | 0.005749631 | 2.50E-12 | 0 | 0.012986566 |
| Genus47 Caproiciproducens | 0.887844082 | 0 | 0.035442475 | 0.064019945 | 0 | 0 | 0.012693498 |
| Genus203 [Bacteroides]_pectinophilus_group | 0.98784414 | 1.54E-13 | 0 | 1.82E-14 | 6.11E-14 | 0 | 0.01215586 |
| Genus45 UCG-010 | 0.755415525 | 0.142803871 | 0.034535048 | 0 | 0.055731791 | 3.05E-12 | 0.011513765 |
| Genus88 Klebsiella | 0.988744896 | 0 | 4.04E-12 | 9.09E-12 | 1.84E-11 | 0 | 0.011255104 |
| Genus14 unknown | 0.988996716 | 1.05E-17 | 0 | 0 | 0 | 4.09E-19 | 0.011003284 |
| Genus117 Coriobacteriaceae_UCG-003 | 0.852593452 | 0.023803801 | 1.24E-11 | 0.114512602 | 0 | 0 | 0.009090145 |
| Genus199 Anaerostipes | 0.978830905 | 4.00E-13 | 0.013242307 | 0 | 0 | 0 | 0.007926788 |
| Genus80 Monoglobus | 0.989848513 | 1.48E-11 | 0.002770011 | 0 | 1.06E-12 | 9.17E-14 | 0.007381476 |
| Genus73 Flavonifractor | 0.826699064 | 0.031706062 | 0.107433527 | 0 | 0.026669337 | 0.000550757 | 0.006941253 |
| Genus13 Dysgonomonas | 0.994126849 | 0 | 7.26E-11 | 0 | 0 | 0 | 0.005873151 |
| Genus43 Tyzzerella | 0.916652513 | 0 | 0 | 0.05769706 | 0.002477042 | 0.017314203 | 0.005859183 |
| Genus115 unknown | 0.956457044 | 0.034517915 | 0 | 0.003273464 | 0 | 0 | 0.005751577 |

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|---|-------------|-------------|-----------------------|-------------|-------------|-------------------------------|--------------|
| Genus195 Megamonas | 0.844684432 | 0.059843799 | 1.42E-13 | 0.081504485 | 0 | 0.008377123 | 0.005590161 |
| Genus180 Peptoclostridium | 0.922253507 | 0.034104539 | 0.038100493 | 0 | 0 | 0 | 0.00554146 |
| Genus58 Paludicola | 0.99448523 | 8.70E-13 | 0 | 0 | 0 | 0 | 0.00551477 |
| Genus34 Shuttleworthia | 0.84782384 | 0.087296619 | 0.021882807 | 0.037756009 | 0 | 0 | 0.005240724 |
| Genus140 Lactobacillus | 0.866030874 | 0.027826131 | 3.45E-11 | 0.050453071 | 0 | 0.051492275 | 0.004197649 |
| Genus130 Libanicoccus | 0.816484362 | 0.025964468 | 1.60E-11 | 0.154586745 | 0 | 5.54E-13 | 0.002964425 |
| Genus61 Hydrogenoanaerobacterium | 0.91150694 | 0.060725083 | 1.77E-13 | 0.002035564 | 0 | 0.022974322 | 0.002758091 |
| Genus110 Victivallis | 0.998099984 | 0 | 6.03E-13 | 0 | 0 | 0 | 0.001900016 |
| Genus145 Asteroleplasma | 0.952961848 | 0.045457767 | 1.06E-11 | 1.10E-10 | 0 | 0 | 0.001580385 |
| Genus65 Ruminococcus | 0.881392296 | 0.007964947 | 5.57E-15 | 0.050794152 | 0.058999868 | 0 | 0.000848737 |
| Genus178 Terrisporobacter | 0.899111136 | 0.080492988 | 0.002062106 | 0 | 0.017799709 | 0 | 0.000534061 |
| Genus172 Family_XIII_UCG-001 | 0.903608878 | 0 | 1.12E-09 | 0.09609108 | 0 | 2.49E-10 | 0.000300041 |
| Genus23 Bacteroides | 0.851075375 | 0.050992231 | 0.061520871 | 2.11E-11 | 0 | 0.036411504 | 1.89E-08 |
| Genus198 [Eubacterium]_hallii_group | 0.883741672 | 0 | 0.005734889 | 4.80E-10 | 0.110523429 | 6.57E-09 | 3.29E-09 |
| Genus81 Clostridia_vadinBB60_group | 0.931930382 | 0.018088692 | 0 | 0.049980921 | 2.23E-09 | 0 | 2.71E-09 |
| Genus97 Comamonas | 0.645286112 | 0.225507082 | 0.102481393 | 0 | 0.026725413 | 0 | 2.45E-10 |
| Genus51 Anaerofilum | 0.963744716 | 0 | 1.26E-09 | 0 | 0 | 0.036255282 | 7.75E-11 |
| Genus171 Mitsukella | 0.969848737 | 0 | 0.007427583 | 0.02272368 | 6.18E-11 | 4.22E-12 | 5.45E-11 |
| Genus109 vadinBE97 | 0.944640321 | 0.053875615 | 0 | 0.001484063 | 0 | 0 | 1.47E-11 |
| Genus185 Defluviitaleaceae_UCG-011 | 0.829563994 | 0.147618405 | 0.005057433 | 0 | 0 | 0.017760168 | 1.32E-11 |
| Genus50 [Eubacterium]_coprostanoligenes_group | 0.816883341 | 0.033276737 | 0.038726107 | 0.027541694 | 0.083572121 | 0 | 1.02E-11 |
| Genus132 Peptococcus | 0.948578208 | 0 | 0.051421792 | 0 | 0 | 0 | 9.66E-12 |
| Genus205 [Eubacterium]_eligens_group | 0.991605742 | 0.008394258 | 7.26E-12 | 0 | 0 | 0 | 6.01E-12 |
| Genus159 Catenibacterium | 0.947026275 | 0.044529157 | 4.27E-11 | 0.008444568 | 0 | 0 | 1.93E-12 |
| Genus118 Gordonibacter | 0.949226681 | 0.012238721 | 0.013940488 | 0.020715122 | 0 | 0.003878988 | 1.72E-12 |
| Genus214 Lachnoclostridium | 0.764808821 | 0 | 0.058693627 | 0 | 0.138682108 | 0.037815444 | 1.65E-12 |
| Genus64 [Eubacterium]_siraeum_group | 0.896499119 | 0.028005438 | 0.009740578 | 0.002894313 | 0.062860553 | 0 | 1.39E-12 |

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|---|-------------|-------------|-----------------------|-------------|-------------|-------------------------------|--------------|
| Genus103 TM7x | 0.851981154 | 0.04613915 | 0.075925005 | 0.02595469 | 0 | 0 | 8.35E-13 |
| Genus158 Coprobacillus | 0.931250049 | 6.41E-12 | 0.02858916 | 0.040160791 | 0 | 0 | 8.27E-13 |
| Genus154 unknown | 0.977763326 | 0 | 0.015286683 | 0.005913299 | 0 | 0.001036692 | 2.07E-13 |
| Genus147 Holdemania | 0.844502363 | 0.028606969 | 0.107509508 | 5.93E-12 | 0 | 0.01938116 | 5.92E-14 |
| Genus16 Prevotella | 0.990329995 | 0 | 0.009670005 | 0 | 0 | 0 | 3.21E-14 |
| Genus201 Eisenbergiella | 0.949458137 | 0.023993382 | 0.026548481 | 0 | 0 | 0 | 2.41E-14 |
| Genus174 Family_XIII_AD3011_group | 0.781327082 | 0.154854338 | 0.021592383 | 0.021799844 | 0 | 0.020426352 | 1.20E-14 |
| Genus128 Enorma | 1 | 0 | 0 | 0 | 0 | 1.58E-18 | 3.92E-17 |
| Genus2 Rikenella | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus3 unknown | 0.912397986 | 0.037024831 | 0.050577183 | 0 | 0 | 0 | 0 |
| Genus4 Rikenellaceae_RC9_gut_group | 0.975662412 | 0.006209009 | 0.018128579 | 0 | 0 | 0 | 0 |
| Genus6 Coprobacter | 0.999522385 | 0 | 0.000477615 | 0 | 0 | 1.86E-12 | 0 |
| Genus8 CAG-873 | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus9 Muribaculaceae | 0.973813527 | 0.026186473 | 2.14E-11 | 0 | 0 | 0 | 0 |
| Genus10 Sanguibacteroides | 0.753907492 | 0.051327402 | 0.096373121 | 0.084282301 | 0 | 0.014109685 | 0 |
| Genus11 Butyricimonas | 0.997402576 | 0 | 0.002597424 | 0 | 2.93E-12 | 1.78E-13 | 0 |
| Genus12 Odoribacter | 0.970077738 | 3.34E-14 | 0.029922262 | 0 | 0 | 0 | 0 |
| Genus15 unknown | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus17 Prevotellaceae_NK3B31_group | 0.984624852 | 0.015375148 | 0 | 4.10E-11 | 2.05E-12 | 8.54E-12 | 0 |
| Genus18 Prevotellaceae_UCG-001 | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus19 Alloprevotella | 0.968337744 | 0.031662256 | 1.89E-12 | 0 | 0 | 7.65E-12 | 0 |
| Genus20 Prevotellaceae_UCG-003 | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus21 Paraprevotella | 0.94099274 | 0 | 7.12E-12 | 0.011844872 | 0.047162388 | 0 | 0 |
| Genus22 Prevotellaceae_Ga6A1_group | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus25 [Ruminococcus]_gavreautii_group | 0.936543066 | 0.063456934 | 2.06E-12 | 0 | 0 | 0 | 0 |
| Genus26 CAG-56 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus27 Marvinbryantia | 0.946527748 | 0.021770476 | 0.024489727 | 0.007212049 | 0 | 4.04E-15 | 0 |

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|--|-------------|-------------|-----------------------|-------------|-------------|-------------------------------|--------------|
| Genus28 Fusicatenibacter | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus30 Sellimonas | 0.892519815 | 0.076534771 | 0.030945413 | 1.17E-12 | 0 | 0 | 0 |
| Genus31 [Ruminococcus]_gnavus_group | 0.934174767 | 0.007677177 | 0.036835973 | 0.021312083 | 1.42E-12 | 0 | 0 |
| Genus32 Agathobacter | 0.995604204 | 0.004395796 | 0 | 4.45E-12 | 3.85E-12 | 0 | 0 |
| Genus33 Roseburia | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus35 Coprococcus | 0.994482355 | 0.005517645 | 9.16E-14 | 0 | 0 | 0 | 0 |
| Genus36 Moryella | 0.999158515 | 0.000841485 | 3.01E-11 | 6.12E-12 | 0 | 0 | 0 |
| Genus37 Blautia | 0.766124029 | 0.093311856 | 0.129240779 | 0 | 1.17E-11 | 0.011323336 | 0 |
| Genus38 Dorea | 0.878387392 | 0.119876211 | 0 | 0.001736397 | 0 | 0 | 0 |
| Genus39 unknown | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus40 [Eubacterium]_fissicatena_group | 0.925921048 | 0.035640358 | 0.038438593 | 1.49E-10 | 0 | 0 | 0 |
| Genus41 Lachnospiraceae_UCG-003 | 0.962089282 | 0 | 0.034674287 | 0 | 0 | 0.003236431 | 0 |
| Genus42 Robinsoniella | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus46 CAG-352 | 0.779016982 | 0.050957329 | 0 | 0.012980097 | 0.070246311 | 0.086799281 | 0 |
| Genus52 UBA1819 | 0.894602587 | 0.044531321 | 0.060866093 | 0 | 0 | 0 | 0 |
| Genus56 Candidatus_Soleaferrea | 0.87166525 | 3.09E-13 | 0 | 0.12833475 | 0 | 0 | 0 |
| Genus57 [Clostridium]_methylpentosum_group | 0.935620783 | 0.049107532 | 3.80E-12 | 0 | 0.006766164 | 0.00850552 | 0 |
| Genus59 Negativibacillus | 0.87434932 | 1.55E-10 | 0.013967311 | 0.017705161 | 0.093978207 | 0 | 0 |
| Genus62 Phoea | 0.905076486 | 0.008138441 | 0.084266951 | 1.81E-13 | 0 | 0.002518121 | 0 |
| Genus66 unknown | 0.989173039 | 0.00203169 | 8.99E-11 | 0 | 0 | 0.008795271 | 0 |
| Genus68 UCG-009 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus69 UCG-005 | 0.785458794 | 0.028822255 | 0.04083225 | 0.01930477 | 0.098078863 | 0.027503069 | 0 |
| Genus70 Intestinimonas | 0.877087898 | 0.039789419 | 7.39E-14 | 0.017214331 | 0.065908352 | 0 | 0 |
| Genus75 Oscillibacter | 0.626475449 | 0.04117133 | 0.162148517 | 0.170204705 | 0 | 0 | 0 |
| Genus76 Oscillospira | 0.869186816 | 0.05478315 | 0 | 0.076030035 | 0 | 0 | 0 |
| Genus77 UCG-003 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus78 UCG-002 | 0.910470252 | 0.00273687 | 0.037357469 | 0 | 0.043423741 | 0.006011668 | 0 |

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|---|-------------|-------------|-----------------------|-------------|-------------|-------------------------------|--------------|
| Genus79 NK4A214_group | 0.839533662 | 0.043208384 | 0.074594353 | 0 | 0 | 0.0426636 | 0 |
| Genus82 Christensenellaceae_R-7_group | 0.726336473 | 0.020391436 | 0.068487916 | 0.035549072 | 0.126493723 | 0.02274138 | 0 |
| Genus83 unknown | 0.999225825 | 0.000774175 | 8.03E-13 | 0 | 0 | 0 | 0 |
| Genus84 Pseudomonas | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus85 Escherichia-Shigella | 0.883355723 | 0.045227632 | 0.009457631 | 2.26E-10 | 0.061959014 | 0 | 0 |
| Genus86 Citrobacter | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus87 Enterobacter | 0.657669789 | 0.001325401 | 0.010919347 | 3.57E-12 | 0.330085462 | 0 | 0 |
| Genus90 Morganella | 0.791807982 | 0.054947284 | 0.093363166 | 0 | 6.81E-12 | 0.059881567 | 0 |
| Genus91 Raoultella | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus92 Hafnia-Obesumbacterium | 0.766787168 | 0.018661819 | 1.72E-09 | 0.084143317 | 0.130407694 | 0 | 0 |
| Genus93 Succinivibrio | 0.984313169 | 0.015686831 | 9.12E-12 | 0 | 4.45E-12 | 0 | 0 |
| Genus94 Haemophilus | 0.904157171 | 0.084053555 | 3.29E-10 | 2.64E-09 | 0 | 0.011789271 | 0 |
| Genus95 Sutterella | 0.969215381 | 0.02143404 | 4.36E-13 | 0 | 1.51E-13 | 0.009350579 | 0 |
| Genus96 Delftia | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus99 Parasutterella | 0.93660067 | 0.036061114 | 1.31E-11 | 0 | 0 | 0.027338217 | 0 |
| Genus100 Acinetobacter | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus101 unknown | 0.78740708 | 0.071155437 | 0.048015656 | 0.01473654 | 0.066115645 | 0.012569641 | 0 |
| Genus102 Elusimicrobium | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus104 Akkermansia | 0.864994496 | 0.04016491 | 0 | 0.026118029 | 0.005848563 | 0.062874002 | 0 |
| Genus106 Bilophila | 0.985420068 | 0.014579932 | 0 | 0 | 0 | 0 | 0 |
| Genus107 Desulfovibrio | 0.705633184 | 0.1608615 | 0.102229348 | 0.031275968 | 1.27E-10 | 0 | 0 |
| Genus108 unknown | 0.870115305 | 0.01801013 | 0.006779346 | 0.050165457 | 0.047898159 | 0.007031603 | 0 |
| Genus114 Gastranaerophilales | 0.963257854 | 0 | 2.63E-12 | 0.003606099 | 0 | 0.033136047 | 0 |
| Genus116 Olsenella | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus119 unknown | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus120 unknown | 0.9010027 | 9.56E-13 | 0.003120111 | 0.067186794 | 0.023872507 | 0.004817888 | 0 |
| Genus121 Enteroscapio | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|---|-------------|-------------|-----------------------|-------------|-------------|-------------------------------|--------------|
| Genus123 Enterorhabdus | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus125 Senegalimassilia | 0.905956775 | 0.084975569 | 2.71E-09 | 0 | 0 | 0.009067653 | 0 |
| Genus126 CHKCI002 | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus127 Slackia | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus133 unknown | 0.989965014 | 0 | 2.14E-12 | 0 | 5.19E-12 | 0.010034986 | 0 |
| Genus134 Anaerofustis | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus135 Cloacibacillus | 0.934934224 | 2.72E-06 | 0.037919592 | 0 | 0 | 0.027143459 | 0 |
| Genus136 DTU014 | 0.939608357 | 0.047527467 | 0.012864176 | 0 | 0 | 1.31E-13 | 0 |
| Genus137 Enterococcus | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus138 Granulicatella | 0.873935736 | 0.057146459 | 0.068917806 | 1.16E-11 | 0 | 0 | 0 |
| Genus139 Pediococcus | 0.861543637 | 0.019736036 | 3.52E-10 | 0.043487427 | 0 | 0.075232899 | 0 |
| Genus141 Lysinibacillus | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus142 Turicibacter | 0.857148743 | 0.054385571 | 0.079978786 | 0 | 0.0084869 | 0 | 0 |
| Genus143 Anaeroplasma | 0.929899173 | 0.070100827 | 0 | 1.91E-12 | 0 | 0 | 0 |
| Genus144 Izemoplasmatales | 0.955285122 | 0.044714878 | 7.85E-13 | 0 | 0 | 7.99E-13 | 0 |
| Genus146 Solobacterium | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus148 Dielma | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus149 Merdibacter | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus151 Faecalibacillus | 0.804281511 | 0.137425879 | 0.032426273 | 0.017520358 | 0.008345979 | 0 | 0 |
| Genus152 Catenisphaera | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus153 Faecalitalea | 0.911824422 | 0.040885051 | 3.40E-14 | 0 | 0.03942781 | 0.007862717 | 0 |
| Genus155 [Clostridium]_innocuum_group | 0.91702618 | 0 | 0.039232004 | 1.21E-10 | 0.042977064 | 0.000764752 | 0 |
| Genus156 Erysipelatoclostridium | 1 | 5.29E-18 | 0 | 0 | 0 | 8.57E-18 | 0 |
| Genus157 Erysipelotrichaceae_UCG-003 | 0.990621544 | 0 | 0.009378456 | 0 | 0 | 0 | 0 |
| Genus160 Fusobacterium | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus161 Phascolarctobacterium | 0.98255711 | 0 | 0.017366573 | 0 | 6.44E-11 | 7.63E-05 | 0 |
| Genus163 Succiniclasticum | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|---|-------------|-------------|-----------------------|-------------|-------------|-------------------------------|--------------|
| Genus164 Veillonella | 0.998191272 | 0 | 4.35E-12 | 0 | 5.92E-11 | 0.001808728 | 0 |
| Genus165 Dialister | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus166 unknown | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus167 Allisonella | 0.95028092 | 0.030566895 | 0.003646351 | 0.015505833 | 4.11E-13 | 0 | 0 |
| Genus168 Megasphaera | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus169 unknown | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus170 Anaerovibrio | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus173 [Eubacterium]_brachy_group | 0.941741237 | 0.031337198 | 0.019107982 | 0.007813582 | 0 | 1.75E-13 | 0 |
| Genus175 Mogibacterium | 0.915039964 | 0 | 0 | 0.084960036 | 4.97E-13 | 0 | 0 |
| Genus181 Sarcina | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus182 Clostridium_sensu_stricto_1 | 0.779220596 | 0.021905281 | 0.111572721 | 0 | 0.062237166 | 0.025064235 | 0 |
| Genus183 Epulopiscium | 0.811671252 | 0.040104032 | 0.090743667 | 0 | 1.43E-13 | 0.057481049 | 0 |
| Genus184 Lachnospiraceae_UCG-010 | 0.985415086 | 0 | 3.96E-11 | 1.93E-11 | 0 | 0.014584914 | 0 |
| Genus186 GCA-900066755 | 0.92820019 | 0 | 0.07179981 | 0 | 0 | 6.72E-10 | 0 |
| Genus187 [Eubacterium]_ventriosum_group | 0.887121541 | 0 | 0.00762595 | 0.105252509 | 0 | 0 | 0 |
| Genus189 Catenibacillus | 0.840321188 | 3.37E-11 | 0.089435254 | 0 | 0 | 0.070243558 | 0 |
| Genus191 Lachnospiraceae_NK4B4_group | 0.997434132 | 0 | 0.002565868 | 0 | 0 | 0 | 0 |
| Genus193 Streptococcus | 0.892341945 | 0.099801758 | 0.001519494 | 3.87E-12 | 0 | 0.006336803 | 0 |
| Genus194 Lachnospiraceae_UCG-001 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus200 Lachnospiraceae_UCG-008 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus204 Lachnospiraceae_ND3007_group | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus206 Lachnospira | 0.937156527 | 0.062843473 | 1.98E-12 | 0 | 0 | 0 | 0 |
| Genus207 CHKCI001 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Genus208 Butyrivibrio | 0.95046196 | 0.022430625 | 7.93E-13 | 0 | 0 | 0.027107415 | 0 |
| Genus210 Lachnospiraceae_NK4A136_group | 0.728088582 | 0.038405192 | 0 | 0.233506226 | 0 | 0 | 0 |
| Genus211 Lachnospiraceae_NK3A20_group | 0.697951556 | 0.086924797 | 0.099043109 | 0.063402343 | 0.030498183 | 0.022180013 | 0 |
| Genus212 Lachnospiraceae_FCS020_group | 0.834513773 | 0.110358344 | 0.004861749 | 0.00431221 | 0.014634087 | 0.031319837 | 0 |

| Genus | Residuals | Age | Fever before baseline | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|----------------------|-----------|-----|-----------------------|-----|----------|-------------------------------|--------------|
| Genus213 UC5-1-2E3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

Supplementary Table 21: Proportion of variation explained by each feature and confounder in the metagenomics subcohort (n=18) at weeks 2 and 4, based on metagenomics data.

| Genus | Residuals | Age | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|---------------------------------|-------------|-------------|-------------|-------------|-------------------------------|--------------|
| g__Butyricimonas | 0.30738084 | 0.350249027 | 0.057785187 | 0.016927827 | 0 | 0.267657118 |
| g__Anaerostipes | 0.461192624 | 0.253978868 | 0 | 0.033979107 | 0.105951007 | 0.144898394 |
| g__Proteobacteria_unclassified | 0.661552511 | 4.86E-12 | 0.198444193 | 1.02E-13 | 0 | 0.140003295 |
| g__Firmicutes_unclassified | 0.828126695 | 1.81E-12 | 0 | 0.034149021 | 0 | 0.137724284 |
| g__Bacteroides | 0.636868332 | 0.188339574 | 0.035813804 | 0.005641197 | 0 | 0.133337093 |
| g__Holdemania | 0.685279454 | 0.12655382 | 2.56E-12 | 9.71E-11 | 0.056705767 | 0.131460959 |
| g__Ruminococcaceae_unclassified | 0.557275475 | 0.09585483 | 0 | 0.0989546 | 0.12076597 | 0.127149125 |
| g__Turicimonas | 0.720913101 | 2.26E-10 | 0.154185551 | 3.05E-10 | 4.30E-10 | 0.124901346 |
| g__Gemmiger | 0.615485211 | 0.151703724 | 0.068848806 | 0.000858058 | 0.038824503 | 0.124279699 |
| g__Coprococcus | 0.533675768 | 0.165693907 | 0.20245534 | 1.58E-11 | 0 | 0.098174985 |
| g__Eisenbergiella | 0.462721768 | 0.196320524 | 0.197897411 | 0.020062346 | 0.032645408 | 0.090352543 |
| g__Methanobrevibacter | 0.800061096 | 1.03E-10 | 0 | 0.115777507 | 0 | 0.084161398 |
| g__Collinsella | 0.774684716 | 8.00E-09 | 0.038056503 | 2.01E-11 | 0.111823074 | 0.075435699 |
| g__Parasutterella | 0.461026522 | 0.056545139 | 0.139584445 | 0.188732143 | 0.080157913 | 0.073953836 |
| g__Holdemanella | 0.530420655 | 3.46E-11 | 0.111059459 | 0.208100639 | 0.086079585 | 0.064339662 |
| g__Bilophila | 0.839166444 | 0 | 0 | 1.89E-11 | 0.096910002 | 0.063923553 |
| g__Gordonibacter | 0.755109769 | 0.124525152 | 0.053922419 | 0 | 0.002869302 | 0.063573358 |
| g__Eggerthella | 0.72188593 | 0.003973232 | 0.021185177 | 0.150091332 | 0.044939751 | 0.057924578 |
| g__Agathobaculum | 0.789447602 | 3.29E-10 | 1.39E-11 | 0.022705837 | 0.13319415 | 0.05465241 |
| g__Lachnoclostridium | 0.782767928 | 0.089957188 | 0.076391683 | 0 | 0 | 0.050883201 |
| g__Coproacter | 0.550099128 | 0.251573095 | 0.135793306 | 0.014887248 | 3.87E-11 | 0.047647223 |
| g__Intestinimonas | 0.772713485 | 0 | 0.04670935 | 0.020896301 | 0.112342894 | 0.047337969 |

| Genus | Residuals | Age | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|-------------------------------------|-------------|-------------|-------------|-------------|-------------------------------|--------------|
| g__Barnesiella | 0.503977944 | 0.025395476 | 0.332009513 | 0.092146078 | 7.21E-12 | 0.046470989 |
| g__Butyrivibrio | 0.541299007 | 0.013009399 | 0.084127195 | 0.227230348 | 0.088137404 | 0.046196647 |
| g__Faecalibacterium | 0.535091668 | 0.226828188 | 0.082111493 | 0.110893477 | 3.24E-11 | 0.045075174 |
| g__Prevotella | 0.816401323 | 0.129053716 | 0.01211533 | 3.86E-12 | 0 | 0.042429632 |
| g__Clostridium | 0.240015051 | 0.608872513 | 0.054168754 | 0.054541685 | 1.36E-10 | 0.042401996 |
| g__Phascolarctobacterium | 0.793611949 | 0.109517083 | 0 | 0.056815706 | 0 | 0.040055261 |
| g__Flavonifractor | 0.72308955 | 0.161511145 | 0.080703008 | 0 | 0 | 0.034696297 |
| g__Romboutsia | 0.896363965 | 0 | 0 | 0.069606769 | 1.28E-14 | 0.034029265 |
| g__Olsenella | 0.82116104 | 0.097263677 | 0.011872612 | 2.60E-12 | 0.039812006 | 0.029890665 |
| g__Dielma | 0.880525456 | 1.52E-12 | 0.070851047 | 0.020980309 | 0 | 0.027643188 |
| g__Erysipelatoclostridium | 0.639786609 | 0.113986394 | 0 | 0.219443293 | 0 | 0.026783704 |
| g__Intestinibacter | 0.592904737 | 0 | 0.055301584 | 0.324218086 | 0.006186171 | 0.021389421 |
| g__Desulfovibrionaceae_unclassified | 0.671764618 | 0.007824942 | 0.077334048 | 0.139627782 | 0.082416883 | 0.021031727 |
| g__Cloacibacillus | 0.499992519 | 0.356517059 | 0.09164589 | 0.030907435 | 2.05E-13 | 0.020937096 |
| g__Enorma | 0.756725516 | 0.079509977 | 0.087212184 | 0.005951716 | 0.05044432 | 0.020156287 |
| g__Acidaminococcus | 0.911133868 | 0.051385486 | 0 | 1.39E-11 | 0.023167049 | 0.014313597 |
| g__Ruminococcus | 0.735187459 | 0.143007345 | 0 | 0 | 0.110937233 | 0.010867964 |
| g__Pseudoflavonifractor | 0.859734708 | 0.052671168 | 0.052140661 | 0 | 0.025763972 | 0.009689491 |
| g__Blastocystis | 0.920043858 | 0.056504221 | 0 | 5.33E-11 | 0.016239134 | 0.007212787 |
| g__Methanospaera | 0.807162572 | 0.179714208 | 0.010823911 | 7.30E-11 | 3.10E-10 | 0.002299308 |
| g__Escherichia | 0.774425769 | 0.075569372 | 0 | 0 | 0.148168293 | 0.001836565 |
| g__Enterococcus | 0.999824609 | 0 | 0 | 0 | 0 | 0.000175391 |
| g__Mitsuokella | 0.988293818 | 2.06E-11 | 2.58E-10 | 0.011706179 | 9.80E-10 | 1.65E-09 |
| g__Sanguibacteroides | 0.336257482 | 0.537288361 | 0.055910909 | 0.070543247 | 0 | 1.30E-09 |
| g__Corynebacterium | 0.881641152 | 0.118358841 | 5.54E-09 | 0 | 0 | 1.17E-09 |
| g__Slackia | 0.787018875 | 0.076359235 | 0.110101635 | 0 | 0.026520254 | 5.96E-11 |
| g__Parabacteroides | 0.768922729 | 0.222868033 | 0 | 0 | 0.008209239 | 5.85E-11 |

| Genus | Residuals | Age | Sex | BMI ≥ 30 | Key COVID-19-related symptoms | Intervention |
|---------------------------------|-------------|-------------|-------------|-------------|-------------------------------|--------------|
| g__Megasphaera | 0.998300816 | 0 | 0 | 0 | 0.001699184 | 1.67E-11 |
| g__Bifidobacterium | 0.291787281 | 0.62260101 | 0 | 0.067589897 | 0.018021812 | 8.05E-12 |
| g__Faecalitalea | 0.552804573 | 0.447195427 | 0 | 0 | 0 | 7.37E-12 |
| g__Hafnia | 0.992705804 | 0 | 0 | 0 | 0.007294196 | 5.25E-12 |
| g__Ruthenibacterium | 0.940839807 | 0.021008059 | 0 | 0.038152135 | 0 | 4.42E-12 |
| g__Lachnospira | 0.88275249 | 0.015614688 | 0 | 0.04064253 | 0.060990292 | 3.38E-12 |
| g__Fusicatenibacter | 0.940654368 | 0.059345632 | 7.82E-12 | 0 | 0 | 1.60E-12 |
| g__Actinomyces | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Propionibacterium | 0.967894073 | 0.014701845 | 1.66E-11 | 0.017404082 | 0 | 0 |
| g__Adlercreutzia | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Asaccharobacter | 0.984679785 | 0.015320215 | 0 | 0 | 0 | 0 |
| g__Odoribacter | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Paraprevotella | 0.91054254 | 0 | 0.035226457 | 2.90E-14 | 0.054231003 | 0 |
| g__Alistipes | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Lactobacillus | 0.756391563 | 0.243608437 | 0 | 0 | 1.74E-11 | 0 |
| g__Pediococcus | 0.996958984 | 2.75E-08 | 0 | 0.003040776 | 2.13E-07 | 0 |
| g__Leuconostoc | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Lactococcus | 0.938336168 | 0.02076716 | 0.040896671 | 2.51E-14 | 0 | 0 |
| g__Streptococcus | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Hungatella | 0.824682047 | 0.086537591 | 0.030565062 | 0 | 0.058215301 | 0 |
| g__Lawsonibacter | 0.617854676 | 0.107533114 | 0.167083796 | 0.107528414 | 5.18E-12 | 0 |
| g__Monoglobus | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Eubacterium | 0.973844521 | 5.27E-09 | 0.026155461 | 1.26E-08 | 0 | 0 |
| g__Anaerotignum | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Blautia | 0.696389297 | 0.292617364 | 0 | 0.010993339 | 9.81E-11 | 0 |
| g__Dorea | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Lachnospiraceae_unclassified | 0.303345671 | 0.316832412 | 0.082985263 | 0.037651839 | 0.259184815 | 0 |

| Genus | Residuals | Age | Sex | BMI \geq 30 | Key COVID-19-related symptoms | Intervention |
|--------------------------|-------------|-------------|-------------|---------------|-------------------------------|--------------|
| g__Roseburia | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Sellimonas | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Tyzzerella | 0.994021108 | 4.54E-15 | 0 | 0.005978892 | 0 | 0 |
| g__Oscillibacter | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Clostridioides | 0.967890786 | 0.014700076 | 1.66E-11 | 0.017409138 | 0 | 0 |
| g__Anaeromassilibacillus | 0.821508686 | 0.073820625 | 0.089101345 | 0 | 0.015569344 | 0 |
| g__Anaerotruncus | 0.96198999 | 0.03801001 | 0 | 3.52E-13 | 0 | 0 |
| g__Faecalibacterium | 0.765835044 | 0.170354396 | 0.06381056 | 1.02E-10 | 2.63E-12 | 0 |
| g__Absiella | 0.952576041 | 0.047423956 | 0 | 0 | 2.33E-09 | 0 |
| g__Catenibacterium | 0.761753166 | 0.027869121 | 0 | 3.28E-15 | 0.210377713 | 0 |
| g__Coprobacillus | 0.947873876 | 5.35E-11 | 0 | 1.90E-10 | 0.052126124 | 0 |
| g__Massiliomicrobiota | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Turicibacter | 0.924214768 | 1.40E-11 | 0.075785232 | 0 | 5.49E-11 | 0 |
| g__Allisonella | 0.979419093 | 0.020580906 | 0 | 7.05E-10 | 0 | 0 |
| g__Dialister | 0.581353008 | 0.382082309 | 0.036564683 | 1.47E-12 | 0 | 0 |
| g__Veillonella | 0.580394263 | 0.232591945 | 0.003874142 | 0.11993764 | 0.06320201 | 0 |
| g__Fusobacterium | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Victivallis | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Sutterella | 0.882013904 | 0.008773242 | 0.077856624 | 0.004816936 | 0.026539294 | 0 |
| g__Citrobacter | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Enterobacter | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Klebsiella | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Haemophilus | 0.878511108 | 0.116351299 | 0 | 0 | 0.005137593 | 0 |
| g__Pseudomonas | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Akkermansia | 1 | 0 | 0 | 0 | 0 | 0 |
| g__Candida | 0.999223525 | 0.000776475 | 0 | 0 | 0 | 0 |
| g__Saccharomyces | 1 | 0 | 0 | 0 | 0 | 0 |

Supplementary Table 22: Maaslin2 output of the significantly differentially abundant pathways found in the nicotinamide vs. placebo comparisons in the metagenomics subcohort (n=18). Only significant features were found at week 2 (FDR<0.25).

| Metacyc ID | Pathway name | Effect size / Beta coefficient | | | | False Discovery Rate | | | |
|-----------------------------|--|--------------------------------|--------|--------|--------|--------------------------|--------|--------|--------|
| | | Placebo vs. nicotinamide | | | | Placebo vs. nicotinamide | | | |
| | | Week 0 | Week 2 | Week 4 | Week 6 | Week 0 | Week 2 | Week 4 | Week 6 |
| AST-PWY | L-arginine degradation II (AST pathway) | -2.7 | -20.4 | 5 | -18.9 | 0.995 | 0.145 | 0.999 | 0.78 |
| BIOTIN-BIOSYNTHESIS-PWY | biotin biosynthesis I | -3.1 | -77.4 | -4.3 | -67.2 | 0.995 | 0.242 | 0.999 | 0.78 |
| COA-PWY-1 | coenzyme A biosynthesis II (mammalian) | 20.2 | 165.8 | 21.4 | 111.1 | 0.995 | 0.132 | 0.999 | 0.782 |
| COA-PWY | coenzyme A biosynthesis I | 12.6 | 116.2 | 8.3 | 76.7 | 0.995 | 0.22 | 0.999 | 0.787 |
| DAPLYSINESYN-PWY | L-lysine biosynthesis I | -44.3 | -70 | 1.9 | -27.1 | 0.995 | 0.175 | 0.999 | 0.852 |
| ECASYN-PWY | enterobacterial common antigen biosynthesis | -3.1 | -8.3 | 1.3 | -8.4 | 0.995 | 0.132 | 0.999 | 0.78 |
| ENTBACSYN-PWY | enterobactin biosynthesis | -5.2 | -58.4 | 4.2 | -34 | 0.995 | 0.132 | 0.999 | 0.78 |
| FAO-PWY | fatty acid β-oxidation I | 4.7 | -96.7 | 10.4 | -69.2 | 0.995 | 0.132 | 0.999 | 0.78 |
| FASYN-ELONG-PWY | fatty acid elongation -- saturated | -9 | -102 | 2.6 | -88.3 | 0.995 | 0.156 | 0.999 | 0.78 |
| FASYN-INITIAL-PWY | superpathway of fatty acid biosynthesis initiation (E. coli) | -3.1 | -108.9 | 4.5 | -93.4 | 0.995 | 0.141 | 0.999 | 0.78 |
| FERMENTATION-PWY | mixed acid fermentation | -8.8 | -57 | 10.9 | -25 | 0.995 | 0.155 | 0.999 | 0.787 |
| FUC-RHAMCAT-PWY | superpathway of fucose and rhamnose degradation | -4.4 | -41.4 | -9.9 | -36 | 0.995 | 0.177 | 0.999 | 0.78 |
| FUCCAT-PWY | fucose degradation | -6.5 | -39.9 | -5.9 | -27.5 | 0.995 | 0.147 | 0.999 | 0.78 |
| GALACTARDEG-PWY | D-galactarate degradation I | -2.5 | -50.9 | 7.9 | -31.3 | 0.995 | 0.132 | 0.999 | 0.78 |
| GLUCARDEG-PWY | D-glucarate degradation I | -0.5 | -32.5 | 3.3 | -24.2 | 0.995 | 0.141 | 0.999 | 0.78 |
| GLUCARGALACTSUPER-PWY | superpathway of D-glucarate and D-galactarate degradation | -2.5 | -50.9 | 7.9 | -31.3 | 0.995 | 0.132 | 0.999 | 0.78 |
| GLUCOSE1PMETAB-PWY | glucose and glucose-1-phosphate degradation | -3.7 | -53.6 | -3.6 | -26.6 | 0.995 | 0.14 | 0.999 | 0.78 |
| GLUDEG-I-PWY | GABA shunt | -10 | -47.5 | -7 | -33.7 | 0.995 | 0.132 | 0.999 | 0.78 |
| GLYCOCAT-PWY | glycogen degradation I (bacterial) | -11.8 | -59.4 | -3.5 | -29.6 | 0.995 | 0.132 | 0.999 | 0.787 |
| GLYCOL-GLYOXDEG-PWY | superpathway of glycol metabolism and degradation | -0.2 | -5.3 | 6.1 | -2 | 0.995 | 0.132 | 0.999 | 0.782 |
| GLYCOLYSIS-TCA-GLYOX-BYPASS | superpathway of glycolysis. pyruvate dehydrogenase. TCA. and glyoxylate bypass | 3.5 | -93.6 | -1.3 | -52.8 | 0.995 | 0.132 | 0.999 | 0.78 |

| Metacyc ID | Pathway name | Effect size / Beta coefficient | | | | False Discovery Rate | | | |
|----------------------|--|--------------------------------|--------|--------|--------|--------------------------|--------|--------|--------|
| | | Placebo vs. nicotinamide | | | | Placebo vs. nicotinamide | | | |
| | | Week 0 | Week 2 | Week 4 | Week 6 | Week 0 | Week 2 | Week 4 | Week 6 |
| GLYOXYLATE-BYPASS | glyoxylate cycle | 0.8 | -49.1 | -1.5 | -23.6 | 0.995 | 0.132 | 0.999 | 0.78 |
| HEME-BIOSYNTHESIS-II | heme biosynthesis I (aerobic) | 3.5 | -29.4 | 3.1 | -19.2 | 0.995 | 0.141 | 0.999 | 0.78 |
| HEMESYN2-PWY | heme biosynthesis II (anaerobic) | 0 | -23 | 1.6 | -9.3 | 0.999 | 0.153 | 0.999 | 0.782 |
| HEXITOLDEGSUPER-PWY | superpathway of hexitol degradation (bacteria) | -15.5 | -73.1 | -10.7 | -37.2 | 0.995 | 0.175 | 0.999 | 0.782 |
| HISTSYN-PWY | L-histidine biosynthesis | 16.6 | 110.2 | -10 | 56.1 | 0.995 | 0.228 | 0.999 | 0.808 |
| KDO-NAGLIPASYN-PWY | superpathway of (Kdo)2-lipid A biosynthesis | -0.8 | -9.4 | 3.4 | -8.7 | 0.995 | 0.147 | 0.999 | 0.78 |
| KETOGLUCONMET-PWY | ketogluconate metabolism | 7 | -43.2 | 7.8 | -24.6 | 0.995 | 0.228 | 0.999 | 0.787 |
| LPSSYN-PWY | superpathway of lipopolysaccharide biosynthesis | -2.5 | -12.5 | -2.3 | -12.3 | 0.995 | 0.242 | 0.999 | 0.78 |
| METH-ACETATE-PWY | methanogenesis from acetate | -6.1 | 14.4 | -13.8 | 11.8 | 0.995 | 0.211 | 0.999 | 0.846 |
| METHGLYUT-PWY | superpathway of methylglyoxal degradation | -6.3 | -27.2 | 0 | -9.8 | 0.995 | 0.14 | 0.999 | 0.787 |
| ORNDEG-PWY | superpathway of ornithine degradation | -16.7 | -48.9 | 4.6 | -28.7 | 0.995 | 0.132 | 0.999 | 0.78 |
| P105-PWY | TCA cycle IV (2-oxoglutarate decarboxylase) | 4.4 | -55.8 | 2.3 | -35.9 | 0.995 | 0.132 | 0.999 | 0.78 |
| P122-PWY | heterolactic fermentation | -1.8 | -13.7 | 3.9 | -12.5 | 0.995 | 0.132 | 0.999 | 0.782 |
| P23-PWY | reductive TCA cycle I | -2.7 | -9.5 | -6.5 | -2.2 | 0.995 | 0.248 | 0.999 | 0.904 |
| P461-PWY | hexitol fermentation to lactate, formate, ethanol and acetate | -17.8 | -79.5 | -11.6 | -38.8 | 0.995 | 0.132 | 0.999 | 0.782 |
| PEPTIDOGLYCANSYN-PWY | peptidoglycan biosynthesis I (meso-diaminopimelate containing) | 46 | 210.3 | 49 | 145.8 | 0.995 | 0.132 | 0.999 | 0.78 |
| POLYAMSYN-PWY | superpathway of polyamine biosynthesis I | -6.3 | -49.3 | 8.7 | -27.2 | 0.995 | 0.14 | 0.999 | 0.782 |
| PWY-1861 | formaldehyde assimilation II (RuMP Cycle) | -13.4 | -58.4 | -14.6 | -22.1 | 0.995 | 0.132 | 0.999 | 0.782 |
| PWY-241 | C4 photosynthetic carbon assimilation cycle, NADP-ME type | -33.1 | -50 | 0 | -24.4 | 0.995 | 0.2 | 0.999 | 0.808 |
| PWY-2723 | trehalose degradation V | -7.1 | -36.2 | -3.1 | -16.9 | 0.995 | 0.132 | 0.999 | 0.808 |
| PWY-3841 | folate transformations II | 28.5 | 155.6 | 24.2 | 82.2 | 0.995 | 0.132 | 0.999 | 0.787 |
| PWY-4041 | gamma-glutamyl cycle | -29.5 | -68.8 | -10.3 | -39.4 | 0.995 | 0.14 | 0.999 | 0.78 |

| Metacyc ID | Pathway name | Effect size / Beta coefficient | | | | False Discovery Rate | | | |
|------------|--|--------------------------------|--------|--------|--------|--------------------------|--------|--------|--------|
| | | Placebo vs. nicotinamide | | | | Placebo vs. nicotinamide | | | |
| | | Week 0 | Week 2 | Week 4 | Week 6 | Week 0 | Week 2 | Week 4 | Week 6 |
| PWY-4242 | pantothenate and coenzyme A biosynthesis III | 9 | 125.6 | 5.7 | 76.1 | 0.995 | 0.23 | 0.999 | 0.787 |
| PWY-4702 | phytate degradation I | -0.4 | -57.7 | -1.1 | -22.9 | 0.995 | 0.132 | 0.999 | 0.782 |
| PWY-5005 | biotin biosynthesis II | 1.8 | 12.2 | 3.9 | -0.4 | 0.995 | 0.175 | 0.999 | 0.99 |
| PWY-5022 | 4-aminobutanoate degradation V | -10.6 | -45 | -7.1 | -33.6 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5083 | NAD/NADH phosphorylation and dephosphorylation | -6.9 | -69.4 | -6.6 | -62.7 | 0.995 | 0.14 | 0.999 | 0.78 |
| PWY-5097 | L-lysine biosynthesis VI | -1.2 | 124.3 | -4.8 | 71.7 | 0.995 | 0.22 | 0.999 | 0.787 |
| PWY-5136 | fatty acid beta-oxidation II (peroxisome) | 5.2 | -82.9 | 12.4 | -66.1 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5138 | unsaturated, even numbered fatty acid beta-oxidation | -3.1 | -18.6 | 11.4 | -22.5 | 0.995 | 0.145 | 0.999 | 0.78 |
| PWY-5173 | superpathway of acetyl-CoA biosynthesis | -3 | -30.5 | 4 | -23.4 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5189 | tetrapyrrole biosynthesis II (from glycine) | -0.1 | -25.7 | 5.1 | -15.4 | 0.995 | 0.14 | 0.999 | 0.784 |
| PWY-5345 | superpathway of L-methionine biosynthesis (by sulphydrylation) | -7.4 | -78.2 | 12.5 | -62.5 | 0.995 | 0.147 | 0.999 | 0.78 |
| PWY-5384 | sucrose degradation IV (sucrose phosphorylase) | -24.4 | -46 | -14.5 | -11.5 | 0.995 | 0.171 | 0.999 | 0.846 |
| PWY-561 | superpathway of glyoxylate cycle and fatty acid degradation | 3.4 | -54.3 | 3.9 | -40.6 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5656 | mannosylglycerate biosynthesis I | -0.9 | -32.8 | 1 | -17.7 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5667 | CDP-diacylglycerol biosynthesis I | 11.8 | 112.7 | -19.1 | 34.5 | 0.995 | 0.23 | 0.999 | 0.904 |
| PWY-5675 | nitrate reduction V (assimilatory) | -3.8 | -54.7 | -0.3 | -30.2 | 0.995 | 0.147 | 0.999 | 0.782 |
| PWY-5676 | acetyl-CoA fermentation to butanoate II | -20.8 | -51.7 | -1 | -24.3 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5686 | UMP biosynthesis | 20.8 | 174.5 | 8.3 | 117.7 | 0.995 | 0.147 | 0.999 | 0.782 |
| PWY-5705 | allantoin degradation to glyoxylate III | -2 | -8.7 | 2.8 | -4 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5723 | Rubisco shunt | -0.5 | -63.1 | 3.1 | -46.2 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5747 | 2-methylcitrate cycle II | -0.6 | -14.9 | 1.9 | -11.7 | 0.995 | 0.228 | 0.999 | 0.782 |
| PWY-5791 | 1,4-dihydroxy-2-naphthoate biosynthesis II (plants) | 0.5 | -36.5 | -1.2 | -27.6 | 0.995 | 0.141 | 0.999 | 0.78 |

| Metacyc ID | Pathway name | Effect size / Beta coefficient | | | | False Discovery Rate | | | |
|------------|---|--------------------------------|--------|--------|--------|--------------------------|--------|--------|--------|
| | | Placebo vs. nicotinamide | | | | Placebo vs. nicotinamide | | | |
| | | Week 0 | Week 2 | Week 4 | Week 6 | Week 0 | Week 2 | Week 4 | Week 6 |
| PWY-5837 | 1,4-dihydroxy-2-naphthoate biosynthesis I | 0.5 | -36.5 | -1.2 | -27.6 | 0.995 | 0.141 | 0.999 | 0.78 |
| PWY-5838 | superpathway of menaquinol-8 biosynthesis I | 0.2 | -70 | -9 | -57.8 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5840 | superpathway of menaquinol-7 biosynthesis | 13.5 | -44.7 | 11.2 | -28.1 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5845 | superpathway of menaquinol-9 biosynthesis | 12.3 | -38.9 | 8 | -34.7 | 0.995 | 0.171 | 0.999 | 0.78 |
| PWY-5850 | superpathway of menaquinol-6 biosynthesis I | 7.4 | -52.9 | 7.2 | -33.3 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5860 | superpathway of demethylmenaquinol-6 biosynthesis I | 5.9 | -40.4 | 7.4 | -25 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5861 | superpathway of demethylmenaquinol-8 biosynthesis | -0.3 | -59.6 | -6.4 | -46.9 | 0.995 | 0.138 | 0.999 | 0.78 |
| PWY-5862 | superpathway of demethylmenaquinol-9 biosynthesis | 9.6 | -30.7 | 8 | -26.4 | 0.995 | 0.176 | 0.999 | 0.78 |
| PWY-5863 | superpathway of phyloquinol biosynthesis | -0.9 | -32.3 | -2.3 | -25.8 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5896 | superpathway of menaquinol-10 biosynthesis | 7.4 | -52.9 | 7.2 | -33.3 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5897 | superpathway of menaquinol-11 biosynthesis | 0.6 | -66.9 | -7.7 | -55 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5898 | superpathway of menaquinol-12 biosynthesis | 0.6 | -66.9 | -7.7 | -55 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5899 | superpathway of menaquinol-13 biosynthesis | 0.6 | -66.9 | -7.7 | -55 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5913 | TCA cycle VI (obligate autotrophs) | -43.2 | -65.9 | -3.9 | -29.4 | 0.995 | 0.153 | 0.999 | 0.795 |
| PWY-5918 | superpathway of heme biosynthesis from glutamate | 4.1 | -36.4 | 3.1 | -23.1 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-5920 | superpathway of heme biosynthesis from glycine | 2.6 | -23.3 | -2.8 | -16.7 | 0.995 | 0.141 | 0.999 | 0.78 |
| PWY-5971 | palmitate biosynthesis II (bacteria and plants) | 5.5 | -105 | 8.1 | -78.3 | 0.995 | 0.141 | 0.999 | 0.78 |
| PWY-5989 | stearate biosynthesis II (bacteria and plants) | -10.6 | -103.4 | 3.7 | -87.5 | 0.995 | 0.141 | 0.999 | 0.78 |
| PWY-6113 | superpathway of mycolate biosynthesis | -2.3 | -67 | 15.1 | -45 | 0.995 | 0.162 | 0.999 | 0.782 |
| PWY-6151 | S-adenosyl-L-methionine cycle I | 16.7 | 160.7 | -3.2 | 142.8 | 0.995 | 0.14 | 0.999 | 0.78 |

| Metacyc ID | Pathway name | Effect size / Beta coefficient | | | | False Discovery Rate | | | |
|------------|--|--------------------------------|--------|--------|--------|--------------------------|--------|--------|--------|
| | | Placebo vs. nicotinamide | | | | Placebo vs. nicotinamide | | | |
| | | Week 0 | Week 2 | Week 4 | Week 6 | Week 0 | Week 2 | Week 4 | Week 6 |
| PWY-6163 | chorismate biosynthesis from 3-dehydroquinate | 12.7 | 140 | 9 | 92.3 | 0.995 | 0.228 | 0.999 | 0.791 |
| PWY-6282 | palmitoleate biosynthesis I (from (5Z)-dodec-5-enoate) | -7 | -102.8 | 5 | -86.3 | 0.995 | 0.141 | 0.999 | 0.78 |
| PWY-6284 | superpathway of unsaturated fatty acids biosynthesis (E. coli) | -2.1 | -25.9 | 11.6 | -19.1 | 0.995 | 0.228 | 0.999 | 0.78 |
| PWY-6285 | superpathway of fatty acids biosynthesis (E. coli) | -4.7 | -53 | 11.7 | -36.9 | 0.995 | 0.14 | 0.999 | 0.78 |
| PWY-6385 | peptidoglycan biosynthesis III (mycobacteria) | 37 | 183.4 | 38.8 | 127.4 | 0.995 | 0.132 | 0.999 | 0.782 |
| PWY-6386 | UDP-N-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing) | 44.8 | 204.8 | 46.2 | 147.7 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-6387 | UDP-N-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing) | 44.7 | 204.8 | 45.7 | 144.2 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-6519 | 8-amino-7-oxononanoate biosynthesis I | -3.7 | -87.3 | 0.1 | -74 | 0.995 | 0.175 | 0.999 | 0.78 |
| PWY-6531 | mannitol cycle | -9.6 | -43.5 | -11.4 | -32.1 | 0.995 | 0.14 | 0.999 | 0.78 |
| PWY-6588 | pyruvate fermentation to acetone | -1.2 | -29.3 | 4.5 | -8.9 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-6609 | adenine and adenosine salvage III | 37.3 | 189.9 | 21.8 | 96.4 | 0.995 | 0.132 | 0.999 | 0.787 |
| PWY-6628 | superpathway of L-phenylalanine biosynthesis | -2.4 | -70.3 | -6.6 | -51.6 | 0.995 | 0.228 | 0.999 | 0.78 |
| PWY-6629 | superpathway of L-tryptophan biosynthesis | 10.9 | -103.4 | -2.9 | -49.2 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-6630 | superpathway of L-tyrosine biosynthesis | -0.9 | -35.4 | -8.8 | -14.1 | 0.995 | 0.155 | 0.999 | 0.795 |
| PWY-6700 | queuosine biosynthesis | 50.3 | 190.4 | 17.6 | 112.9 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-6731 | starch degradation III | -7.9 | -34.1 | -3.6 | -17.2 | 0.995 | 0.132 | 0.999 | 0.805 |
| PWY-6803 | phosphatidylcholine acyl editing | 0.8 | -40 | 0.5 | -18 | 0.995 | 0.132 | 0.999 | 0.787 |
| PWY-6823 | molybdenum cofactor biosynthesis | -0.4 | -8.1 | 6.6 | -4.3 | 0.995 | 0.179 | 0.999 | 0.782 |
| PWY-6837 | fatty acid beta-oxidation V (unsaturated, odd number, di-isomerase-dependent) | -0.8 | -5.7 | 2.8 | -9.2 | 0.995 | 0.242 | 0.999 | 0.78 |
| PWY-6969 | TCA cycle V (2-oxoglutarate-ferredoxin oxidoreductase) | 7.6 | -36.7 | 0.7 | -24.1 | 0.995 | 0.246 | 0.999 | 0.782 |

| Metacyc ID | Pathway name | Effect size / Beta coefficient | | | | False Discovery Rate | | | |
|------------|---|--------------------------------|--------|--------|--------|--------------------------|--------|--------|--------|
| | | Placebo vs. nicotinamide | | | | Placebo vs. nicotinamide | | | |
| | | Week 0 | Week 2 | Week 4 | Week 6 | Week 0 | Week 2 | Week 4 | Week 6 |
| PWY-7013 | L-1,2-propanediol degradation | -13 | -26.9 | 8.3 | -12.3 | 0.995 | 0.221 | 0.999 | 0.78 |
| PWY-7046 | 4-coumarate degradation | -4.2 | -17.8 | -2.6 | -8.1 | 0.995 | 0.144 | 0.999 | 0.78 |
| PWY-7094 | (anaerobic) fatty acid salvage | 1.9 | -15.9 | 2.6 | -14.2 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-7117 | C4 photosynthetic carbon | -49.2 | -64.8 | -9.9 | -31.1 | 0.995 | 0.177 | 0.999 | 0.793 |
| PWY-7199 | assimilation cycle. PEPCK type pyrimidine | 13.5 | 111.1 | -1.2 | 51.6 | 0.995 | 0.132 | 0.999 | 0.846 |
| PWY-7204 | deoxyribonucleosides salvage pyridoxal 5'-phosphate salvage | -2.8 | -24.2 | 2.8 | -16.7 | 0.995 | 0.14 | 0.999 | 0.78 |
| PWY-7219 | II (plants) adenosine ribonucleotides de | 18.9 | 183.2 | 28.2 | 109.6 | 0.995 | 0.132 | 0.999 | 0.782 |
| PWY-7221 | novo biosynthesis guanosine ribonucleotides de | 5.6 | 156 | 9.2 | 101.8 | 0.995 | 0.147 | 0.999 | 0.787 |
| PWY-7254 | novo biosynthesis TCA cycle VII (acetate- producers) | 8 | -53.3 | 5.5 | -28.4 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY-7328 | superpathway of UDP-glucose- derived O-antigen building | -5.6 | -37.6 | -3.2 | -18.5 | 0.995 | 0.132 | 0.999 | 0.787 |
| PWY-7388 | blocks biosynthesis octanoyl-[acyl-carrier protein] biosynthesis (mitochondria, yeast) | -5.8 | -108.4 | 4.9 | -93.4 | 0.995 | 0.144 | 0.999 | 0.78 |
| PWY-7664 | oleate biosynthesis IV (anaerobic) | -8.9 | -101.9 | 3.4 | -86.3 | 0.995 | 0.147 | 0.999 | 0.78 |
| PWY-821 | superpathway of sulfur amino acid biosynthesis (<i>Saccharomyces cerevisiae</i>) | -11.2 | -43.8 | 2.2 | -27.4 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY0-1298 | superpathway of pyrimidine deoxyribonucleosides degradation | -2.5 | -37.4 | 8.7 | -31 | 0.995 | 0.248 | 0.999 | 0.78 |
| PWY0-1319 | CDP-diacylglycerol biosynthesis II | 11.8 | 112.7 | -19.1 | 34.5 | 0.995 | 0.23 | 0.999 | 0.904 |
| PWY0-1415 | superpathway of heme biosynthesis from uroporphyrinogen-III | 3.4 | -27 | 0.4 | -14.4 | 0.995 | 0.132 | 0.999 | 0.78 |
| PWY0-1533 | methylphosphonate degradation I | -1.7 | -2.6 | 0.3 | -4.7 | 0.995 | 0.242 | 0.999 | 0.782 |
| PWY0-42 | 2-methylcitrate cycle I | -0.6 | -15.6 | 2.3 | -11.5 | 0.995 | 0.21 | 0.999 | 0.78 |
| PWY0-862 | (5Z)-dodec-5-enoate biosynthesis | -8.8 | -101.1 | 4.6 | -85.6 | 0.995 | 0.141 | 0.999 | 0.78 |

| Metacyc ID | Pathway name | Effect size / Beta coefficient | | | | False Discovery Rate | | | |
|-------------------|--|--------------------------------|--------|--------|--------|--------------------------|--------|--------|--------|
| | | Placebo vs. nicotinamide | | | | Placebo vs. nicotinamide | | | |
| | | Week 0 | Week 2 | Week 4 | Week 6 | Week 0 | Week 2 | Week 4 | Week 6 |
| PWY30-355 | stearate biosynthesis III (fungi) | -2.7 | -13.2 | 0.7 | -9.4 | 0.995 | 0.14 | 0.999 | 0.78 |
| PWY66-391 | fatty acid beta-oxidation VI (peroxisome) | 2 | -13 | -0.1 | -16.6 | 0.995 | 0.248 | 0.999 | 0.782 |
| PWYG-321 | mycolate biosynthesis | -10.1 | -106.4 | 2.7 | -97.7 | 0.995 | 0.147 | 0.999 | 0.78 |
| PYRIDNUCSAL-PWY | NAD salvage pathway I | -25.5 | -73.1 | -1.4 | -33.9 | 0.995 | 0.141 | 0.999 | 0.787 |
| REDCITCYC | TCA cycle VIII (helicobacter) | 2 | -9.3 | -4.6 | -7 | 0.995 | 0.175 | 0.999 | 0.78 |
| RIBOSYN2-PWY | flavin biosynthesis I (bacteria and plants) | 33.7 | 132.8 | -2.8 | 75.4 | 0.995 | 0.167 | 0.999 | 0.787 |
| SO4ASSIM-PWY | sulfate reduction I (assimilatory) | -8.9 | -78.1 | 17.9 | -57.1 | 0.995 | 0.14 | 0.999 | 0.78 |
| SULFATE-CYS-PWY | superpathway of sulfate assimilation and cysteine biosynthesis | -7.1 | -93.3 | 15.1 | -73.7 | 0.995 | 0.14 | 0.999 | 0.78 |
| TCA-GLYOX-BYPASS | superpathway of glyoxylate bypass and TCA | -1.3 | -68.4 | 0.2 | -39.2 | 0.995 | 0.132 | 0.999 | 0.78 |
| TRNA-CHARGING-PWY | tRNA charging | 44.3 | 185.6 | 57.2 | 130.9 | 0.995 | 0.132 | 0.999 | 0.78 |
| ARG+POLYAMINE-SYN | superpathway of arginine and polyamine biosynthesis | -5.3 | -62.8 | 12.7 | -36.2 | 0.995 | 0.147 | 0.999 | 0.782 |
| 1CMET2-PWY | N-formyl-tetrahydrofolate biosynthesis | 30.8 | 111.5 | -1.8 | 51.2 | 0.995 | 0.195 | 0.999 | 0.846 |

Supplementary Table 23: Maaslin2 output of overlapping pathways between the Essex et al. cohort (n=38) and the COVit-2 metagenomics subcohort (n=18).

| Metacyc ID | Pathway Name | Metadata | value | coef | stderr | pval | name | qval | N | N.not. zero | model |
|-----------------------------|---|--------------|---------|------------|------------|------------|------------------|------------|----|----------------|-------------------------|
| PWY0-1298 | PWY0-1298: superpathway of pyrimidine deoxyribonucleosides degradation | Intervention | Placebo | 37.4099348 | 16.5001362 | 0.04186536 | InterventionPLB | 0.24755878 | 18 | 17 | NAMvsPLB_W2 |
| METHGLYUT-PWY | METHGLYUT-PWY: superpathway of methylglyoxal degradation | OSCI_Class | Severe | 13.8658885 | 5.28596317 | 0.01349321 | OSCI_ClassSevere | 0.24478599 | 38 | 34 | CtrlvsOSCI_Class_Visit1 |
| PWY-6385 | PWY-6385: peptidoglycan biosynthesis III (mycobacteria) | OSCI_Class | Severe | -109.19865 | 41.6363584 | 0.01335622 | OSCI_ClassSevere | 0.24477325 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-5345 | PWY-5345: superpathway of L-methionine biosynthesis (by sulphydrylation) | OSCI_Class | Severe | 80.4284709 | 30.4813101 | 0.01276326 | OSCI_ClassSevere | 0.24403195 | 38 | 35 | CtrlvsOSCI_Class_Visit1 |
| SO4ASSIM-PWY | SO4ASSIM-PWY: sulfate reduction I (assimilatory) | OSCI_Class | Severe | 55.4883109 | 20.9663651 | 0.01251874 | OSCI_ClassSevere | 0.24403195 | 38 | 35 | CtrlvsOSCI_Class_Visit1 |
| SULFATE-CYS-PWY | SULFATE-CYS-PWY: superpathway of sulfate assimilation and cysteine biosynthesis | OSCI_Class | Severe | 77.0732927 | 29.2732912 | 0.01300097 | OSCI_ClassSevere | 0.24403195 | 38 | 35 | CtrlvsOSCI_Class_Visit1 |
| TRNA-CHARGING-PWY | TRNA-CHARGING-PWY: tRNA charging | OSCI_Class | Severe | -127.7557 | 48.6714578 | 0.0131799 | OSCI_ClassSevere | 0.24403195 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-5862 | PWY-5862: superpathway of demethylmenaquinol-9 biosynthesis | OSCI_Class | Severe | 33.3786446 | 12.5352202 | 0.01202663 | OSCI_ClassSevere | 0.2431695 | 38 | 29 | CtrlvsOSCI_Class_Visit1 |
| PWY-3841 | PWY-3841: folate transformations II | OSCI_Class | Severe | -86.313336 | 31.9223219 | 0.01097873 | OSCI_ClassSevere | 0.23474927 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-5675 | PWY-5675: nitrate reduction V (assimilatory) | OSCI_Class | Severe | 47.3074943 | 17.4865551 | 0.01091362 | OSCI_ClassSevere | 0.23474927 | 38 | 23 | CtrlvsOSCI_Class_Visit1 |
| RIBOSYN2-PWY | RIBOSYN2-PWY: flavin biosynthesis I (bacteria and plants) | OSCI_Class | Mild | -76.075471 | 28.0410439 | 0.01064365 | OSCI_ClassMild | 0.23474927 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY0-1298 | PWY0-1298: superpathway of pyrimidine deoxyribonucleosides degradation | OSCI_Class | Mild | 34.966509 | 13.0070595 | 0.00977496 | OSCI_ClassMild | 0.2343015 | 81 | 81 | Longitudinal |
| GALACTARDEG-PWY | GALACTARDEG-PWY: D-galactarate degradation I | OSCI_Class | Severe | 56.1704457 | 19.1040268 | 0.00605283 | OSCI_ClassSevere | 0.23059394 | 38 | 30 | CtrlvsOSCI_Class_Visit1 |
| GLUCARDEG-PWY | GLUCARDEG-PWY: D-glucarate degradation I | OSCI_Class | Severe | 35.049727 | 12.180839 | 0.00715019 | OSCI_ClassSevere | 0.23059394 | 38 | 30 | CtrlvsOSCI_Class_Visit1 |
| GLUCARGALACTSUPER-PWY | GLUCARGALACTSUPER-PWY: superpathway of D-glucarate and D-galactarate degradation | OSCI_Class | Severe | 56.1704457 | 19.1040268 | 0.00605283 | OSCI_ClassSevere | 0.23059394 | 38 | 30 | CtrlvsOSCI_Class_Visit1 |
| GLYCOLYSIS-TCA-GLYOX-BYPASS | GLYCOLYSIS-TCA-GLYOX-BYPASS: superpathway of glycolysis. pyruvate dehydrogenase. TCA. and glyoxylate bypass | OSCI_Class | Severe | 23.9617966 | 8.67747713 | 0.00953505 | OSCI_ClassSevere | 0.23059394 | 38 | 12 | CtrlvsOSCI_Class_Visit1 |
| PWY-5747 | PWY-5747: 2-methylcitrate cycle II | OSCI_Class | Severe | 7.03222378 | 2.4531806 | 0.00728244 | OSCI_ClassSevere | 0.23059394 | 38 | 13 | CtrlvsOSCI_Class_Visit1 |
| PWY-5837 | PWY-5837: 1,4-dihydroxy-2-naphthoate biosynthesis I | OSCI_Class | Severe | 28.3332032 | 10.3734966 | 0.01017749 | OSCI_ClassSevere | 0.23059394 | 38 | 33 | CtrlvsOSCI_Class_Visit1 |
| PWY-5838 | PWY-5838: superpathway of menaquinol-8 biosynthesis I | OSCI_Class | Severe | 51.7483401 | 18.2192064 | 0.00777294 | OSCI_ClassSevere | 0.23059394 | 38 | 32 | CtrlvsOSCI_Class_Visit1 |
| PWY-5845 | PWY-5845: superpathway of menaquinol-9 biosynthesis | OSCI_Class | Severe | 44.0044157 | 15.9779145 | 0.00962409 | OSCI_ClassSevere | 0.23059394 | 38 | 29 | CtrlvsOSCI_Class_Visit1 |

| Metacyc ID | Pathway Name | Metadata | value | coef | stderr | pval | name | qval | N | N.not. zero | model |
|----------------------|--|--------------|---------|------------|------------|------------|------------------|------------|----|----------------|-------------------------|
| PWY-5861 | PWY-5861: superpathway of demethylmenaquinol-8 biosynthesis | OSCI_Class | Severe | 40.497192 | 14.6560182 | 0.00941087 | OSCI_ClassSevere | 0.23059394 | 38 | 32 | CtrlvsOSCI_Class_Visit1 |
| PWY-5897 | PWY-5897: superpathway of menaquinol-11 biosynthesis | OSCI_Class | Severe | 50.5862896 | 17.8387885 | 0.00786203 | OSCI_ClassSevere | 0.23059394 | 38 | 32 | CtrlvsOSCI_Class_Visit1 |
| PWY-5898 | PWY-5898: superpathway of menaquinol-12 biosynthesis | OSCI_Class | Severe | 50.5862896 | 17.8387885 | 0.00786203 | OSCI_ClassSevere | 0.23059394 | 38 | 32 | CtrlvsOSCI_Class_Visit1 |
| PWY-5899 | PWY-5899: superpathway of menaquinol-13 biosynthesis | OSCI_Class | Severe | 50.5862896 | 17.8387885 | 0.00786203 | OSCI_ClassSevere | 0.23059394 | 38 | 32 | CtrlvsOSCI_Class_Visit1 |
| PWY-7254 | PWY-7254: TCA cycle VII (acetate-producers) | OSCI_Class | Severe | 9.33241814 | 3.38281607 | 0.01015915 | OSCI_ClassSevere | 0.23059394 | 38 | 22 | CtrlvsOSCI_Class_Visit1 |
| REDCITCYC | REDCITCYC: TCA cycle VIII (helicobacter) | OSCI_Class | Severe | 16.8746978 | 5.72216538 | 0.0063237 | OSCI_ClassSevere | 0.23059394 | 38 | 30 | CtrlvsOSCI_Class_Visit1 |
| HISTSYN-PWY | HISTSYN-PWY: L-histidine biosynthesis | Intervention | Placebo | -110.18472 | 46.643097 | 0.03567061 | InterventionPLB | 0.22829187 | 18 | 18 | NAMvsPLB_W2 |
| PWY-5747 | PWY-5747: 2-methylcitrate cycle II | Intervention | Placebo | 14.8989671 | 6.28389986 | 0.03451748 | InterventionPLB | 0.2281287 | 18 | 9 | NAMvsPLB_W2 |
| PWY-6163 | PWY-6163: chorismate biosynthesis from 3-dehydroquinate | Intervention | Placebo | -139.99202 | 59.4614654 | 0.03492687 | InterventionPLB | 0.2281287 | 18 | 17 | NAMvsPLB_W2 |
| HEME-BIOSYNTHESIS-II | HEME-BIOSYNTHESIS-II: heme biosynthesis I (aerobic) | OSCI_Class | Severe | 31.7228249 | 10.065858 | 0.00351443 | OSCI_ClassSevere | 0.22392838 | 38 | 37 | CtrlvsOSCI_Class_Visit1 |
| PEPTIDOGLYCANSYN-PWY | PEPTIDOGLYCANSYN-PWY: peptidoglycan biosynthesis I (meso-diaminopimelate containing) | OSCI_Class | Severe | -126.51023 | 42.3095803 | 0.00538255 | OSCI_ClassSevere | 0.22392838 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-1861 | PWY-1861: formaldehyde assimilation II (RuMP Cycle) | OSCI_Class | Severe | -49.210176 | 16.1113202 | 0.00451898 | OSCI_ClassSevere | 0.22392838 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-5840 | PWY-5840: superpathway of menaquinol-7 biosynthesis | OSCI_Class | Severe | 47.118169 | 15.7129105 | 0.00521761 | OSCI_ClassSevere | 0.22392838 | 38 | 32 | CtrlvsOSCI_Class_Visit1 |
| PWY-6386 | PWY-6386: UDP-N-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing) | OSCI_Class | Severe | -140.30826 | 46.3484817 | 0.004876 | OSCI_ClassSevere | 0.22392838 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-6387 | PWY-6387: UDP-N-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing) | OSCI_Class | Severe | -133.67513 | 44.7162142 | 0.00537206 | OSCI_ClassSevere | 0.22392838 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-5097 | PWY-5097: L-lysine biosynthesis VI | Intervention | Placebo | -124.33038 | 52.77046 | 0.032493 | InterventionPLB | 0.22029149 | 18 | 17 | NAMvsPLB_W2 |
| PWY-6700 | PWY-6700: queuosine biosynthesis | OSCI_Class | Severe | -121.62666 | 37.9507924 | 0.00317492 | OSCI_ClassSevere | 0.21119095 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-6151 | PWY-6151: S-adenosyl-L-methionine cycle I | OSCI_Class | Severe | -167.09189 | 48.9554323 | 0.00723467 | OSCI_ClassSevere | 0.20748352 | 81 | 81 | Longitudinal |
| HISTSYN-PWY | HISTSYN-PWY: L-histidine biosynthesis | OSCI_Class | Mild | -85.997104 | 30.52032 | 0.00709674 | OSCI_ClassMild | 0.20691198 | 81 | 81 | Longitudinal |
| PWY-6163 | PWY-6163: chorismate biosynthesis from 3-dehydroquinate | OSCI_Class | Mild | -83.459458 | 29.3668035 | 0.00700081 | OSCI_ClassMild | 0.20633377 | 81 | 81 | Longitudinal |
| TRNA-CHARGING-PWY | TRNA-CHARGING-PWY: tRNA charging | OSCI_Class | Mild | -74.659177 | 26.7237321 | 0.00691649 | OSCI_ClassMild | 0.20608873 | 81 | 81 | Longitudinal |

| Metacyc ID | Pathway Name | Metadata | value | coef | stderr | pval | name | qval | N | N.not. zero | model |
|----------------------|--|--------------|---------|------------|------------|------------|------------------|------------|----|----------------|-------------------------|
| KDO-NAGLIPASYN-PWY | KDO-NAGLIPASYN-PWY: superpathway of (Kdo)2-lipid A biosynthesis | OSCI_Class | Severe | 6.85139742 | 2.12431062 | 0.00289818 | OSCI_ClassSevere | 0.20019726 | 38 | 12 | CtrlvsOSCI_Class_Visit1 |
| PWY-6151 | PWY-6151: S-adenosyl-L-methionine cycle I | OSCI_Class | Mild | -98.415087 | 34.4756118 | 0.00617243 | OSCI_ClassMild | 0.18923586 | 81 | 81 | Longitudinal |
| PWY-5097 | PWY-5097: L-lysine biosynthesis VI | OSCI_Class | Severe | -120.20356 | 36.8193294 | 0.00261235 | OSCI_ClassSevere | 0.18767122 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-7221 | PWY-7221: guanosine ribonucleotides de novo biosynthesis | OSCI_Class | Severe | -123.52293 | 40.7050094 | 0.00587824 | OSCI_ClassSevere | 0.18641915 | 81 | 81 | Longitudinal |
| PWY-5686 | PWY-5686: UMP biosynthesis | OSCI_Class | Severe | -182.36579 | 40.1441212 | 0.00560823 | OSCI_ClassSevere | 0.17996102 | 81 | 81 | Longitudinal |
| PWY-5862 | PWY-5862: superpathway of demethylmenaquinol-9 biosynthesis | Intervention | Placebo | 30.6504653 | 11.9056594 | 0.02391452 | InterventionPLB | 0.17551941 | 18 | 16 | NAMvsPLB_W2 |
| PWY-5686 | PWY-5686: UMP biosynthesis | OSCI_Class | Mild | -85.296629 | 29.0286723 | 0.00513939 | OSCI_ClassMild | 0.17491429 | 81 | 81 | Longitudinal |
| REDCITCYC | REDCITCYC: TCA cycle VIII (helicobacter) | Intervention | Placebo | 9.32562113 | 3.69097672 | 0.02325092 | InterventionPLB | 0.17464776 | 18 | 12 | NAMvsPLB_W2 |
| PWY-5845 | PWY-5845: superpathway of menaquinol-9 biosynthesis | Intervention | Placebo | 38.8863992 | 14.8597031 | 0.02206677 | InterventionPLB | 0.17139239 | 18 | 16 | NAMvsPLB_W2 |
| PWY-6163 | PWY-6163: chorismate biosynthesis from 3-dehydroquinone | OSCI_Class | Severe | -160.13387 | 44.1169067 | 0.00453931 | OSCI_ClassSevere | 0.16746044 | 81 | 81 | Longitudinal |
| RIBOSYN2-PWY | RIBOSYN2-PWY: flavin biosynthesis I (bacteria and plants) | Intervention | Placebo | -132.7633 | 51.5491279 | 0.02110374 | InterventionPLB | 0.16715832 | 18 | 17 | NAMvsPLB_W2 |
| PWY-6151 | PWY-6151: S-adenosyl-L-methionine cycle I | OSCI_Class | Severe | -174.09261 | 51.0092188 | 0.00176305 | OSCI_ClassSevere | 0.16588355 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-6163 | PWY-6163: chorismate biosynthesis from 3-dehydroquinone | OSCI_Class | Severe | -161.39829 | 47.0920648 | 0.00174696 | OSCI_ClassSevere | 0.16588355 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-6609 | PWY-6609: adenine and adenosine salvage III | OSCI_Class | Severe | -144.29214 | 42.8666343 | 0.00205883 | OSCI_ClassSevere | 0.16588355 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| HEMESYN2-PWY | HEMESYN2-PWY: heme biosynthesis II (anaerobic) | Intervention | Placebo | 23.0241131 | 8.4873752 | 0.0183393 | InterventionPLB | 0.15282749 | 18 | 17 | NAMvsPLB_W2 |
| KDO-NAGLIPASYN-PWY | KDO-NAGLIPASYN-PWY: superpathway of (Kdo)2-lipid A biosynthesis | Intervention | Placebo | 9.40481141 | 3.40724048 | 0.01690677 | InterventionPLB | 0.1470868 | 18 | 10 | NAMvsPLB_W2 |
| PWY-5345 | PWY-5345: superpathway of L-methionine biosynthesis (by sulfhydrylation) | Intervention | Placebo | 78.2412898 | 28.2849817 | 0.0166289 | InterventionPLB | 0.1470868 | 18 | 17 | NAMvsPLB_W2 |
| PWY-5675 | PWY-5675: nitrate reduction V (assimilatory) | Intervention | Placebo | 54.7066298 | 19.7253425 | 0.01640906 | InterventionPLB | 0.1470868 | 18 | 12 | NAMvsPLB_W2 |
| PWY-5686 | PWY-5686: UMP biosynthesis | Intervention | Placebo | -174.47422 | 64.4880043 | 0.01627726 | InterventionPLB | 0.1470868 | 18 | 17 | NAMvsPLB_W2 |
| PWY-7221 | PWY-7221: guanosine ribonucleotides de novo biosynthesis | Intervention | Placebo | -155.97834 | 58.094458 | 0.01696418 | InterventionPLB | 0.1470868 | 18 | 18 | NAMvsPLB_W2 |
| GLUCARDEG-PWY | GLUCARDEG-PWY: D-glucarate degradation I | Intervention | Placebo | 32.5348383 | 11.3651748 | 0.0139434 | InterventionPLB | 0.1411257 | 18 | 13 | NAMvsPLB_W2 |
| HEME-BIOSYNTHESIS-II | HEME-BIOSYNTHESIS-II: heme biosynthesis I (aerobic) | Intervention | Placebo | 29.4235404 | 10.2367076 | 0.01361252 | InterventionPLB | 0.1411257 | 18 | 17 | NAMvsPLB_W2 |

| Metacyc ID | Pathway Name | Metadata | value | coef | stderr | pval | name | qval | N | N.not. zero | model |
|-----------------------------|---|--------------|---------|------------|------------|------------|------------------|------------|----|----------------|--------------|
| PWY-5837 | PWY-5837: 1,4-dihydroxy-2-naphthoate biosynthesis I | Intervention | Placebo | 36.4887406 | 12.8063722 | 0.01428898 | InterventionPLB | 0.1411257 | 18 | 16 | NAMvsPLB_W2 |
| PYRIDNUCSAL-PWY | PYRIDNUCSAL-PWY: NAD salvage pathway I | Intervention | Placebo | 73.144394 | 26.219424 | 0.01374221 | InterventionPLB | 0.1411257 | 18 | 17 | NAMvsPLB_W2 |
| METHGLYUT-PWY | METHGLYUT-PWY: superpathway of methylglyoxal degradation | Intervention | Placebo | 27.246507 | 9.27733463 | 0.01207246 | InterventionPLB | 0.13962377 | 18 | 17 | NAMvsPLB_W2 |
| PWY-6151 | PWY-6151: S-adenosyl-L-methionine cycle I | Intervention | Placebo | -160.66872 | 55.2736857 | 0.01084442 | InterventionPLB | 0.13962377 | 18 | 17 | NAMvsPLB_W2 |
| SO4ASSIM-PWY | SO4ASSIM-PWY: sulfate reduction I (assimilatory) | Intervention | Placebo | 78.0781031 | 26.0761247 | 0.01075156 | InterventionPLB | 0.13962377 | 18 | 17 | NAMvsPLB_W2 |
| SULFATE-CYS-PWY | SULFATE-CYS-PWY: superpathway of sulfate assimilation and cysteine biosynthesis | Intervention | Placebo | 93.2602457 | 31.2324564 | 0.01091073 | InterventionPLB | 0.13962377 | 18 | 17 | NAMvsPLB_W2 |
| PWY-3841 | PWY-3841: folate transformations II | OSCI_Class | Severe | -95.534361 | 25.3503453 | 0.00335088 | OSCI_ClassSevere | 0.13766514 | 81 | 81 | Longitudinal |
| PWY-5861 | PWY-5861: superpathway of demethylmenaquinol-8 biosynthesis | Intervention | Placebo | 59.6472691 | 19.5702179 | 0.00980529 | InterventionPLB | 0.13761805 | 18 | 16 | NAMvsPLB_W2 |
| COA-PWY-1 | COA-PWY-1: coenzyme A biosynthesis II (mammalian) | Intervention | Placebo | -165.78645 | 53.9111621 | 0.00769766 | InterventionPLB | 0.1321481 | 18 | 18 | NAMvsPLB_W2 |
| GALACTARDEG-PWY | GALACTARDEG-PWY: D-galactarate degradation I | Intervention | Placebo | 50.8573341 | 15.3857294 | 0.00610934 | InterventionPLB | 0.1321481 | 18 | 14 | NAMvsPLB_W2 |
| GLUCARGALACTSUPER-PWY | GLUCARGALACTSUPER-PWY: superpathway of D-glucarate and D-galactarate degradation | Intervention | Placebo | 50.8573341 | 15.3857294 | 0.00610934 | InterventionPLB | 0.1321481 | 18 | 14 | NAMvsPLB_W2 |
| GLYCOLYSIS-TCA-GLYOX-BYPASS | GLYCOLYSIS-TCA-GLYOX-BYPASS: superpathway of glycolysis. pyruvate dehydrogenase. TCA. and glyoxylate bypass | Intervention | Placebo | 93.58483 | 27.4113497 | 0.00485441 | InterventionPLB | 0.1321481 | 18 | 10 | NAMvsPLB_W2 |
| P461-PWY | P461-PWY: hexitol fermentation to lactate, formate, ethanol and acetate | Intervention | Placebo | 79.5284894 | 23.5169072 | 0.00516443 | InterventionPLB | 0.1321481 | 18 | 17 | NAMvsPLB_W2 |
| PEPTIDOGLYCANSYN-PWY | PEPTIDOGLYCANSYN-PWY: peptidoglycan biosynthesis I (meso-diaminopimelate containing) | Intervention | Placebo | -210.26714 | 53.1362643 | 0.00126501 | InterventionPLB | 0.1321481 | 18 | 17 | NAMvsPLB_W2 |
| PWY-1861 | PWY-1861: formaldehyde assimilation II (RuMP Cycle) | Intervention | Placebo | 58.4274854 | 17.6403696 | 0.00558204 | InterventionPLB | 0.1321481 | 18 | 16 | NAMvsPLB_W2 |
| PWY-3841 | PWY-3841: folate transformations II | Intervention | Placebo | -155.64632 | 45.3162395 | 0.003686 | InterventionPLB | 0.1321481 | 18 | 18 | NAMvsPLB_W2 |
| PWY-5838 | PWY-5838: superpathway of menaquinol-8 biosynthesis I | Intervention | Placebo | 70.0437239 | 22.679501 | 0.00904325 | InterventionPLB | 0.1321481 | 18 | 16 | NAMvsPLB_W2 |
| PWY-5840 | PWY-5840: superpathway of menaquinol-7 biosynthesis | Intervention | Placebo | 44.6758256 | 14.5329233 | 0.00925037 | InterventionPLB | 0.1321481 | 18 | 15 | NAMvsPLB_W2 |
| PWY-5897 | PWY-5897: superpathway of menaquinol-11 biosynthesis | Intervention | Placebo | 66.8765235 | 21.6941825 | 0.00914145 | InterventionPLB | 0.1321481 | 18 | 16 | NAMvsPLB_W2 |
| PWY-5898 | PWY-5898: superpathway of menaquinol-12 biosynthesis | Intervention | Placebo | 66.8765235 | 21.6941825 | 0.00914145 | InterventionPLB | 0.1321481 | 18 | 16 | NAMvsPLB_W2 |
| PWY-5899 | PWY-5899: superpathway of menaquinol-13 biosynthesis | Intervention | Placebo | 66.8765235 | 21.6941825 | 0.00914145 | InterventionPLB | 0.1321481 | 18 | 16 | NAMvsPLB_W2 |

| Metacyc ID | Pathway Name | Metadata | value | coef | stderr | pval | name | qval | N | N.not. zero | model |
|-----------------------|--|--------------|---------|------------|------------|------------|------------------|------------|----|----------------|-------------------------|
| PWY-6385 | PWY-6385: peptidoglycan biosynthesis III (mycobacteria) | Intervention | Placebo | -183.38051 | 55.1606787 | 0.00462102 | InterventionPLB | 0.1321481 | 18 | 17 | NAMvsPLB_W2 |
| PWY-6386 | PWY-6386: UDP-N-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing) | Intervention | Placebo | -204.84427 | 54.7119214 | 0.0019546 | InterventionPLB | 0.1321481 | 18 | 17 | NAMvsPLB_W2 |
| PWY-6387 | PWY-6387: UDP-N-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing) | Intervention | Placebo | -204.81418 | 53.8723519 | 0.00173668 | InterventionPLB | 0.1321481 | 18 | 17 | NAMvsPLB_W2 |
| PWY-6609 | PWY-6609: adenine and adenosine salvage III | Intervention | Placebo | -189.90436 | 48.1064036 | 0.00128983 | InterventionPLB | 0.1321481 | 18 | 18 | NAMvsPLB_W2 |
| PWY-6700 | PWY-6700: queuosine biosynthesis | Intervention | Placebo | -190.44389 | 52.8328035 | 0.00260073 | InterventionPLB | 0.1321481 | 18 | 17 | NAMvsPLB_W2 |
| PWY-7254 | PWY-7254: TCA cycle VII (acetate-producers) | Intervention | Placebo | 53.3077718 | 15.0818748 | 0.0039111 | InterventionPLB | 0.1321481 | 18 | 10 | NAMvsPLB_W2 |
| TRNA-CHARGING-PWY | TRNA-CHARGING-PWY: tRNA charging | Intervention | Placebo | -185.62854 | 53.3461342 | 0.00336065 | InterventionPLB | 0.1321481 | 18 | 17 | NAMvsPLB_W2 |
| RIBOSYN2-PWY | RIBOSYN2-PWY: flavin biosynthesis I (bacteria and plants) | OSCI_Class | Severe | -93.198681 | 28.8209267 | 0.00270549 | OSCI_ClassSevere | 0.11764008 | 81 | 81 | Longitudinal |
| P461-PWY | P461-PWY: hexitol fermentation to lactate, formate, ethanol and acetate | OSCI_Class | Severe | 100.361386 | 30.8078532 | 0.00231188 | OSCI_ClassSevere | 0.10808037 | 81 | 81 | Longitudinal |
| HEMESYN2-PWY | HEMESYN2-PWY: heme biosynthesis II (anaerobic) | OSCI_Class | Severe | 40.7776722 | 10.4873294 | 0.00049377 | OSCI_ClassSevere | 0.10619678 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-5686 | PWY-5686: UMP biosynthesis | OSCI_Class | Severe | -166.88799 | 44.8950782 | 0.00077193 | OSCI_ClassSevere | 0.10619678 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PWY-7221 | PWY-7221: guanosine ribonucleotides de novo biosynthesis | OSCI_Class | Severe | -156.79178 | 37.9834431 | 0.0002486 | OSCI_ClassSevere | 0.10619678 | 38 | 38 | CtrlvsOSCI_Class_Visit1 |
| PEPTIDOGLYCAN SYN-PWY | PEPTIDOGLYCAN SYN-PWY: peptidoglycan biosynthesis I (meso-diaminopimelate containing) | OSCI_Class | Severe | -146.69463 | 37.3505936 | 0.00225046 | OSCI_ClassSevere | 0.10612364 | 81 | 81 | Longitudinal |
| PWY-6387 | PWY-6387: UDP-N-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing) | OSCI_Class | Severe | -153.12316 | 41.3172769 | 0.00206664 | OSCI_ClassSevere | 0.0991806 | 81 | 81 | Longitudinal |
| PWY-6386 | PWY-6386: UDP-N-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing) | OSCI_Class | Severe | -158.38076 | 43.2082438 | 0.00193128 | OSCI_ClassSevere | 0.09880497 | 81 | 81 | Longitudinal |
| RIBOSYN2-PWY | RIBOSYN2-PWY: flavin biosynthesis I (bacteria and plants) | OSCI_Class | Mild | -73.948307 | 22.1599036 | 0.00160839 | OSCI_ClassMild | 0.08846376 | 81 | 81 | Longitudinal |
| COA-PWY-1 | COA-PWY-1: coenzyme A biosynthesis II (mammalian) | OSCI_Class | Severe | -132.95548 | 36.204371 | 0.00084129 | OSCI_ClassSevere | 0.06525202 | 81 | 81 | Longitudinal |
| METHGLYUT-PWY | METHGLYUT-PWY: superpathway of methylglyoxal degradation | OSCI_Class | Severe | 17.2698114 | 4.79670869 | 0.00085023 | OSCI_ClassSevere | 0.06525202 | 81 | 73 | Longitudinal |
| PWY-6700 | PWY-6700: queuosine biosynthesis | OSCI_Class | Severe | -123.66623 | 33.0083041 | 0.00070764 | OSCI_ClassSevere | 0.06091353 | 81 | 81 | Longitudinal |
| PWY0-1298 | PWY0-1298: superpathway of pyrimidine deoxyribonucleosides degradation | OSCI_Class | Severe | 64.7373965 | 15.5071111 | 0.00057161 | OSCI_ClassSevere | 0.05879199 | 81 | 81 | Longitudinal |
| TRNA-CHARGING-PWY | TRNA-CHARGING-PWY: tRNA charging | OSCI_Class | Severe | -146.73103 | 38.1097349 | 0.00059158 | OSCI_ClassSevere | 0.05879199 | 81 | 81 | Longitudinal |

Supplementary Table 24: Mean variance contribution of patients' covariates at baseline.

| Covariate | Variance mean |
|-------------------------------------|---------------|
| Residuals | 0.888 |
| Fever before baseline | 0.031 |
| BMI | 0.028 |
| Age | 0.027 |
| Other key COVID-19-related symptoms | 0.017 |
| Sex | 0.009 |

Data from 34 patients receiving nicotinamide and 30 receiving placebo.

2.5. Safety data

Supplementary Table 25: Adverse events sorted by MedDRA System Organ Class and Preferred Term.

| System Organ Class | Preferred Term | Complaint / adverse event | Nicotinamide | | | Placebo | | | Total | | | Fisher exact test |
|--------------------------------------|-----------------------------|--|--------------|--------|--------|----------|--------|--------|----------|--------|--------|-------------------|
| | | | Patients | % | Events | Patients | % | Events | Patients | % | Events | P value** |
| Any System Organ Class | All* | | 317 | 70.76% | 1798 | 297 | 65.71% | 1732 | 614 | 68.22% | 3530 | 0.115 |
| Blood and lymphatic system disorders | All | | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Lymphadenopathy | Swollen lymph nodes | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| Cardiac disorders | All | | 12 | 2.68% | 12 | 9 | 1.99% | 9 | 21 | 2.33% | 21 | 0.517 |
| | Angina pectoris | Angina pectoris | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Arrhythmia | Cardiac arrhythmia | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |
| | Cardiovascular disorder | Circulatory problems | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Chest pain | Heart pain | 0 | 0.00% | 0 | 3 | 0.66% | 3 | 3 | 0.33% | 3 | 0.249 |
| | Myocarditis | Myocarditis | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Peripheral swelling | Swollen leg | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | | Swollen leg: possible post-thrombotic syndrome | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Tachycardia | Tachycardia | 7 | 1.56% | 7 | 3 | 0.66% | 3 | 10 | 1.11% | 10 | 0.222 |
| Ear and labyrinth disorders | All | | 11 | 2.46% | 11 | 12 | 2.65% | 12 | 23 | 2.56% | 23 | >0.999 |
| | Ear pain | Ear pain | 3 | 0.67% | 3 | 1 | 0.22% | 1 | 4 | 0.44% | 4 | 0.372 |
| | Eustachian tube dysfunction | Plugged ears | 0 | 0.00% | 0 | 6 | 1.33% | 6 | 6 | 0.67% | 6 | 0.031 |
| | External ear inflammation | Ear canal inflammation | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Hyperacusis | Noise sensitivity | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Hypoacusis | Impaired hearing | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |
| | Middle ear effusion | Middle ear effusion | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Otitis media | Otitis media | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Tinnitus | Tinnitus | 3 | 0.67% | 3 | 3 | 0.66% | 3 | 6 | 0.67% | 6 | >0.999 |
| Endocrine disorders | Vertigo | Transient vertigo | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | All | | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Thyroiditis subacute | Viral thyroiditis | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| Eye disorders | All | | 11 | 2.46% | 11 | 3 | 0.66% | 3 | 14 | 1.56% | 14 | 0.033 |
| | Blepharospasm | Eye twitching | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |

| System Organ Class | Preferred Term | Complaint / adverse event | Nicotinamide | | | Placebo | | | Total | | | Fisher exact test |
|--------------------------------|----------------------------------|---|--------------|--------|--------|----------|--------|--------|----------|--------|--------|-------------------|
| | | | Patients | % | Events | Patients | % | Events | Patients | % | Events | P value** |
| Eye disorders (<i>cont.</i>) | Conjunctivitis | Conjunctivitis | 6 | 1.34% | 6 | 0 | 0.00% | 0 | 6 | 0.67% | 6 | 0.015 |
| | Dry eye | Dry eyes | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Visual impairment | Impaired vision | 3 | 0.67% | 3 | 2 | 0.44% | 2 | 5 | 0.56% | 5 | 0.685 |
| Gastrointestinal disorders | All | | 113 | 25.22% | 113 | 80 | 17.70% | 82 | 193 | 21.44% | 195 | 0.007 |
| | Abdominal distension | Bloating | 2 | 0.45% | 2 | 3 | 0.66% | 3 | 5 | 0.56% | 5 | >0.999 |
| | Abdominal pain | Abdominal pain | 10 | 2.23% | 10 | 6 | 1.33% | 6 | 16 | 1.78% | 16 | 0.326 |
| | Breath odor | Bad breath | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Constipation | Constipation | 4 | 0.89% | 4 | 2 | 0.44% | 2 | 6 | 0.67% | 6 | 0.450 |
| | Diarrhea | Diarrhea | 10 | 2.23% | 10 | 10 | 2.21% | 11 | 20 | 2.22% | 21 | >0.999 |
| | | Increased diarrhea | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Dyspepsia | Heartburn | 3 | 0.67% | 3 | 2 | 0.44% | 2 | 5 | 0.56% | 5 | 0.685 |
| | Dysphagia | Difficulty swallowing the trial tablets | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |
| | | Dysphagia | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Eructation | Belching | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Feces discolored | Stool color changes | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Feces hard | Hard stool | 3 | 0.67% | 3 | 0 | 0.00% | 0 | 3 | 0.33% | 3 | 0.123 |
| | Feces soft | Soft stool | 6 | 1.34% | 6 | 1 | 0.22% | 1 | 7 | 0.78% | 7 | 0.068 |
| | Flatulence | Flatulence | 2 | 0.45% | 2 | 3 | 0.66% | 3 | 5 | 0.56% | 5 | >0.999 |
| | Gastritis | Gastritis | 0 | 0.00% | 0 | 2 | 0.44% | 2 | 2 | 0.22% | 2 | 0.499 |
| | Gastrointestinal disorder | Digestive problems | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Gastrointestinal sounds abnormal | Bowel sounds | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | | Stomach noises | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |
| | Gastroesophageal reflux disease | Increased reflux | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Gingival bleeding | Bleeding gums | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Nausea | Nausea | 8 | 1.79% | 8 | 7 | 1.55% | 7 | 15 | 1.67% | 15 | 0.801 |
| | Noninfective gingivitis | Inflamed gums | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Pulpitis dental | Inflamed tooth | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Taste disorder | Impaired sense of taste | 48 | 10.71% | 48 | 40 | 8.85% | 41 | 88 | 9.78% | 89 | 0.370 |
| | Tongue coated | Coated tongue | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |
| | Tongue discomfort | Burning sensation on the tongue | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |

| System Organ Class | Preferred Term | Complaint / adverse event | Nicotinamide | | | Placebo | | | Total | | | Fisher exact test |
|--|---------------------------------------|---|--------------|--------|--------|----------|--------|--------|----------|--------|--------|-------------------|
| | | | Patients | % | Events | Patients | % | Events | Patients | % | Events | P value** |
| Gastrointestinal disorders (<i>cont.</i>) | Vomiting | Vomiting | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| General disorders and administration site conditions | All | | 111 | 24.78% | 122 | 109 | 24.12% | 118 | 220 | 24.44% | 240 | 0.877 |
| | Adverse reaction | Unspecified side effects | 2 | 0.45% | 2 | 2 | 0.44% | 2 | 4 | 0.44% | 4 | >0.999 |
| | Chest pain | Chest pain | 13 | 2.90% | 13 | 16 | 3.54% | 17 | 29 | 3.22% | 30 | 0.707 |
| | Chills | Chills | 2 | 0.45% | 2 | 1 | 0.22% | 1 | 3 | 0.33% | 3 | 0.623 |
| | Decreased activity | Performance drop (reduced physical performance) | 51 | 11.38% | 58 | 33 | 7.30% | 34 | 84 | 9.33% | 92 | 0.039 |
| | Facial pain | Pain in the face and jaws | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Fatigue | Fatigue | 18 | 4.02% | 19 | 23 | 5.09% | 23 | 41 | 4.56% | 42 | 0.523 |
| | General physical health deterioration | Reduced ability to perform normal activities | 15 | 3.35% | 17 | 17 | 3.76% | 22 | 32 | 3.56% | 39 | 0.858 |
| | Hot flush | Hot flashes | 3 | 0.67% | 3 | 1 | 0.22% | 1 | 4 | 0.44% | 4 | 0.372 |
| | Malaise | General feeling of sickness / malaise | 2 | 0.45% | 3 | 6 | 1.33% | 8 | 8 | 0.89% | 11 | 0.287 |
| | Mucosal disorder | Dry mucous membranes (respiratory tract) | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Night sweats | Night sweats | 2 | 0.45% | 2 | 1 | 0.22% | 1 | 3 | 0.33% | 3 | 0.623 |
| | Peripheral coldness | Cold fingers | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | | Cold hands and feet | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Pyrexia | Fever | 1 | 0.22% | 1 | 3 | 0.66% | 3 | 4 | 0.44% | 4 | 0.624 |
| | Sensation of foreign body | Lump in throat | 1 | 0.22% | 1 | 2 | 0.44% | 2 | 3 | 0.33% | 3 | >0.999 |
| Infections and infestations | All | | 8 | 1.79% | 8 | 14 | 3.10% | 14 | 22 | 2.44% | 22 | 0.280 |
| | Appendicitis | Suspected appendicitis | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | COVID-19 | Death by COVID-19 | 0 | 0.00% | 0 | 0 | 0.00% | 0 | 0 | 0.00% | 0 | >0.999 |
| | Herpes zoster | Shingles | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Pharyngitis | Pharyngitis | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |
| | Pneumonia | Pneumonia | 4 | 0.89% | 4 | 7 | 1.55% | 7 | 11 | 1.22% | 11 | 0.546 |
| | Sinusitis | Sinusitis | 1 | 0.22% | 1 | 4 | 0.88% | 4 | 5 | 0.56% | 5 | 0.374 |
| | Tonsillitis | Tonsillitis | 0 | 0.00% | 0 | 2 | 0.44% | 2 | 2 | 0.22% | 2 | 0.499 |
| Investigations | All | | 16 | 3.57% | 16 | 15 | 3.32% | 15 | 31 | 3.44% | 31 | 0.857 |
| | Blood pressure decreased | Decreased blood pressure | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Blood pressure increased | Increased blood pressure | 1 | 0.22% | 1 | 2 | 0.44% | 2 | 3 | 0.33% | 3 | >0.999 |

| System Organ Class | Preferred Term | Complaint / adverse event | Nicotinamide | | | Placebo | | | Total | | | Fisher exact test |
|---|----------------------------|--|--------------|--------|--------|----------|--------|--------|----------|--------|--------|-------------------|
| | | | Patients | % | Events | Patients | % | Events | Patients | % | Events | P value** |
| Investigations (<i>cont.</i>) | Blood test abnormal | Deteriorated blood parameters | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Emergency care examination | Examination in an emergency department without hospitalization | 8 | 1.79% | 8 | 7 | 1.55% | 7 | 15 | 1.67% | 15 | 0.801 |
| | Heart rate increased | Elevated heart rate (resting) | 3 | 0.67% | 3 | 2 | 0.44% | 2 | 5 | 0.56% | 5 | 0.685 |
| | | Elevated heart rate (under stress) | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Hepatic enzyme increased | Increased liver enzymes | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Respiratory rate increased | Increased respiratory rate | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Tidal volume decreased | Reduced lung volume | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |
| Metabolism and nutrition disorders | All | | 8 | 1.79% | 9 | 3 | 0.66% | 3 | 11 | 1.22% | 12 | 0.142 |
| | Alcohol intolerance | Reduced alcohol tolerance | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Decreased appetite | Loss of appetite / lower food intake | 6 | 1.34% | 7 | 3 | 0.66% | 3 | 9 | 1.00% | 10 | 0.339 |
| | Increased appetite | Increased appetite | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| Musculoskeletal and connective tissue disorders | All | | 35 | 7.81% | 35 | 28 | 6.19% | 28 | 63 | 7.00% | 63 | 0.363 |
| | Arthralgia | Joint pain | 9 | 2.01% | 9 | 8 | 1.77% | 8 | 17 | 1.89% | 17 | 0.812 |
| | Back pain | Back pain | 3 | 0.67% | 3 | 3 | 0.66% | 3 | 6 | 0.67% | 6 | >0.999 |
| | Myalgia | Muscle pain | 13 | 2.90% | 13 | 14 | 3.10% | 14 | 27 | 3.00% | 27 | >0.999 |
| | Neck pain | Neck pain | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Pain in extremity | Finger pain | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | | Foot pain | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | | Limb pain | 7 | 1.56% | 7 | 2 | 0.44% | 2 | 9 | 1.00% | 9 | 0.106 |
| Nervous system disorders | All | | 73 | 16.29% | 74 | 75 | 16.59% | 75 | 148 | 16.44% | 149 | 0.928 |
| | Aphasia | Word finding difficulties | 4 | 0.89% | 4 | 7 | 1.55% | 7 | 11 | 1.22% | 11 | 0.546 |
| | Balance disorder | Balance disorders | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Dizziness | Dizziness | 22 | 4.91% | 23 | 18 | 3.98% | 18 | 40 | 4.44% | 41 | 0.522 |
| | Headache | Headache | 31 | 6.92% | 31 | 32 | 7.08% | 32 | 63 | 7.00% | 63 | >0.999 |
| | Memory impairment | Memory problems | 12 | 2.68% | 12 | 15 | 3.32% | 15 | 27 | 3.00% | 27 | 0.697 |
| | Migraine | Migraine | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Neuralgia | Nerve pain (arm, hand) | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |

| System Organ Class | Preferred Term | Complaint / adverse event | Nicotinamide | | | Placebo | | | Total | | | Fisher exact test |
|---|--|---------------------------|--------------|--------|--------|----------|--------|--------|----------|--------|--------|-------------------|
| | | | Patients | % | Events | Patients | % | Events | Patients | % | Events | P value** |
| Nervous system disorders (<i>cont.</i>) | Neuralgia (<i>cont.</i>) | Nerve pain (arm) | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | | Nerve pain (leg) | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Paraesthesia | Tingling in the abdomen | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Restless legs syndrome | Restless legs | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| Psychiatric disorders | All | | 99 | 22.10% | 122 | 112 | 24.78% | 147 | 211 | 23.44% | 269 | 0.346 |
| | Apathy | Motivation problems | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Confusional state | Confusion | 9 | 2.01% | 9 | 11 | 2.43% | 11 | 20 | 2.22% | 20 | 0.822 |
| | Depression | Depression | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Disturbance in attention | Concentration problems | 25 | 5.58% | 25 | 24 | 5.31% | 24 | 49 | 5.44% | 49 | 0.884 |
| | Irritability | Irritability | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Nervousness | Nervousness | 0 | 0.00% | 0 | 2 | 0.44% | 2 | 2 | 0.22% | 2 | 0.499 |
| | Panic attack | Panic attacks | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Restlessness | Restlessness | 0 | 0.00% | 0 | 2 | 0.44% | 2 | 2 | 0.22% | 2 | 0.499 |
| | Sleep disorder | Disturbed sleep | 62 | 13.84% | 85 | 70 | 15.49% | 105 | 132 | 14.67% | 190 | 0.510 |
| | Reproductive system and breast disorders | All | 1 | 0.22% | 1 | 2 | 0.44% | 2 | 3 | 0.33% | 3 | >0.999 |
| | | Intermenstrual bleeding | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | | Menstrual disorder | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| Respiratory, thoracic and mediastinal disorders | Menstruation irregular | Irregular menstrual cycle | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | All | | 368 | 82.14% | 404 | 368 | 81.42% | 405 | 736 | 81.78% | 809 | 0.796 |
| | Bronchial disorder | Mucus in the bronchi | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Bronchitis | Bronchitis | 1 | 0.22% | 1 | 2 | 0.44% | 2 | 3 | 0.33% | 3 | >0.999 |
| | Chest discomfort | Chest tightness | 7 | 1.56% | 7 | 5 | 1.11% | 5 | 12 | 1.33% | 12 | 0.577 |
| | Cough | Cough | 59 | 13.17% | 63 | 65 | 14.38% | 67 | 124 | 13.78% | 130 | 0.629 |
| | | Severe cough | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Dysphonia | Hoarseness | 7 | 1.56% | 7 | 10 | 2.21% | 11 | 17 | 1.89% | 18 | 0.626 |
| | Dyspnea | Shortness of breath | 135 | 30.13% | 162 | 121 | 26.77% | 139 | 256 | 28.44% | 301 | 0.269 |
| | Hyposmia | Impaired sense of smell | 58 | 12.95% | 59 | 52 | 11.50% | 54 | 110 | 12.22% | 113 | 0.542 |
| | Nasal discomfort | Cold nose | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Nasal dryness | Dry nose | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Oropharyngeal pain | Sore throat | 21 | 4.69% | 21 | 17 | 3.76% | 18 | 38 | 4.22% | 39 | 0.512 |

| System Organ Class | Preferred Term | Complaint / adverse event | Nicotinamide | | | Placebo | | | Total | | | Fisher exact test |
|--|---------------------------------|--|--------------|-------|--------|----------|-------|--------|----------|-------|--------|-------------------|
| | | | Patients | % | Events | Patients | % | Events | Patients | % | Events | P value** |
| Respiratory, thoracic and mediastinal disorders (cont.) | Productive cough | Cough with sputum production | 28 | 6.25% | 29 | 20 | 4.42% | 21 | 48 | 5.33% | 50 | 0.238 |
| | Respiratory tract congestion | Congested sensation in the respiratory tract | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Respiratory tract disorders | Mucus production in the respiratory tract | 19 | 4.24% | 22 | 36 | 7.96% | 47 | 57 | 6.33% | 69 | 0.025 |
| | Respiratory tract irritation | Lung irritation | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Rhinorrhea | Rhinitis/rhinorrhea | 21 | 4.69% | 21 | 28 | 6.19% | 29 | 49 | 5.44% | 50 | 0.378 |
| | Sinus congestion | Sinus congestion | 1 | 0.22% | 1 | 3 | 0.66% | 3 | 4 | 0.44% | 4 | 0.624 |
| | Throat clearing | Frequent throat clearing | 2 | 0.45% | 2 | 1 | 0.22% | 1 | 3 | 0.33% | 3 | 0.623 |
| | Wheezing | Whistling/wheezing breathing | 7 | 1.56% | 7 | 4 | 0.88% | 4 | 11 | 1.22% | 11 | 0.383 |
| Skin and subcutaneous tissue disorders | All | | 26 | 5.80% | 26 | 29 | 6.42% | 29 | 55 | 6.11% | 55 | 0.781 |
| | Alopecia | Hair loss | 10 | 2.23% | 10 | 10 | 2.21% | 10 | 20 | 2.22% | 20 | >0.999 |
| | Dermatitis atopic | Atopic dermatitis flare | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Dry skin | Dry skin | 2 | 0.45% | 2 | 1 | 0.22% | 1 | 3 | 0.33% | 3 | 0.623 |
| | Hyperhidrosis | Sweats | 1 | 0.22% | 1 | 3 | 0.66% | 3 | 4 | 0.44% | 4 | 0.624 |
| | Pruritus | Pruritus | 1 | 0.22% | 1 | 1 | 0.22% | 1 | 2 | 0.22% | 2 | >0.999 |
| | Rash | Skin rash | 10 | 2.23% | 10 | 12 | 2.65% | 12 | 22 | 2.44% | 22 | 0.830 |
| | Skin discoloration | Discolored toes | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| | Skin discomfort | Skin discomfort (face) | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | | Skin discomfort (legs) | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| Social circumstances | All | | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Overwork | Overwhelmed at work | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| Surgical and medical procedures | All | | 4 | 0.89% | 4 | 4 | 0.88% | 4 | 8 | 0.89% | 8 | >0.999 |
| | Hospitalization | Hospitalization | 4 | 0.89% | 4 | 3 | 0.66% | 3 | 7 | 0.78% | 7 | 0.724 |
| | Hospitalization, oxygen therapy | Hospitalization with a continuous oxygen requirement of more than 24 hours | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Intensive care | Intensive care requirement | 0 | 0.00% | 0 | 0 | 0.00% | 0 | 0 | 0.00% | 0 | >0.999 |
| | Mechanical ventilation | Ventilation requirement | 0 | 0.00% | 0 | 0 | 0.00% | 0 | 0 | 0.00% | 0 | >0.999 |
| Vascular disorders | All | | 2 | 0.45% | 2 | 0 | 0.00% | 0 | 2 | 0.22% | 2 | 0.248 |
| | Hematoma | Hematomas | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |

| System Organ Class | Preferred Term | Complaint / adverse event | Nicotinamide | | | Placebo | | | Total | | | Fisher exact test |
|--|------------------------|--|--------------|-------|--------|----------|-------|--------|----------|-------|--------|-------------------|
| | | | Patients | % | Events | Patients | % | Events | Patients | % | Events | P value** |
| Vascular disorders (<i>cont.</i>) | Varicose vein ruptured | Recurrence of anal varicose vein rupture | 1 | 0.22% | 1 | 0 | 0.00% | 0 | 1 | 0.11% | 1 | 0.498 |
| Vision disorders | All | | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |
| | Photopsia | Flashes of light in the eyes | 0 | 0.00% | 0 | 1 | 0.22% | 1 | 1 | 0.11% | 1 | >0.999 |

The safety population (n=900) consisted of 448 patients assigned to receive nicotinamide and 452 patients assigned to receive placebo.

* As some patients reported AEs from multiple System Organ Classes, the total number of patients in the summary line “System Organ Class: Any System Organ Class; Preferred Term: All” is not identical to the total number of patients from all individual System Organ Classes / Preferred Terms.

** Unadjusted P values, two-sided, from exploratory analyses. None of the tests remains statistically significant after applying corrections for multiple testing.

Supplementary Table 26: Severe COVID-19, Serious Adverse Events and emergency treatment in the safety population at week 0 (baseline).

| Events / status at week 0 | Nicotinamide n=448 | | Placebo n=452 | | Total n=900 |
|---|-----------------------|--------|------------------|--------|----------------|
| | n | % | n | % | n |
| Missing | 0 | 0.00 | 0 | 0.00 | 0 |
| WHO Scale (current status at week 0 interview) | | | | | |
| 0 – No clinical or virological evidence of infection | 0 | 0.00 | 0 | 0.00 | 0 |
| 1 – No limitation of activities | 65 | 14.51 | 70 | 15.49 | 135 |
| 2 – Limitation of activities | 383 | 85.49 | 382 | 84.51 | 765 |
| 3 – Hospitalized, no oxygen therapy | 0 | 0.00 | 0 | 0.00 | 0 |
| 4 – Oxygen by mask or nasal prongs | 0 | 0.00 | 0 | 0.00 | 0 |
| 5 – Non-invasive ventilation or high-flow oxygen | 0 | 0.00 | 0 | 0.00 | 0 |
| 6 – Intubation and mechanical ventilation | 0 | 0.00 | 0 | 0.00 | 0 |
| 7 – Ventilation + additional organ support – pressors, RRT, ECMO | 0 | 0.00 | 0 | 0.00 | 0 |
| 8 – Death | 0 | 0.00 | 0 | 0.00 | 0 |
| Severe COVID-19 (before week 0 [Baseline]) | | | | | |
| Examination in an emergency department without hospitalization | 2 | 0.45 | 0 | 0.00 | 2 |
| Hospitalization | 0 | 0.00 | 0 | 0.00 | 0 |
| Hospitalization with a continuous oxygen requirement of ≥24 hours | 0 | 0.00 | 0 | 0.00 | 0 |
| Intensive care requirement | 0 | 0.00 | 0 | 0.00 | 0 |
| Ventilation requirement | 0 | 0.00 | 0 | 0.00 | 0 |
| Death by COVID-19 | 0 | 0.00 | 0 | 0.00 | 0 |
| Duration of hospitalization [days] | | | | | |
| 0 | 448 | 100.00 | 452 | 100.00 | 900 |

Supplementary Table 27: Severe COVID-19, Serious Adverse Events and emergency treatment in the safety population at week 2.

| Events / status at week 2 | Nicotinamide n=448 | | Placebo n=452 | | Total n=900 |
|---|-----------------------|-------|------------------|-------|----------------|
| | n | % | n | % | n |
| Missing | 31 | 6.92 | 27 | 5.97 | 58 |
| WHO Scale (current status at week 2 interview) | | | | | |
| 0 – No clinical or virological evidence of infection | 0 | 0.00 | 1 | 0.24 | 1 |
| 1 – No limitation of activities | 233 | 55.88 | 222 | 52.24 | 455 |
| 2 – Limitation of activities | 184 | 44.12 | 201 | 47.29 | 385 |
| 3 – Hospitalized, no oxygen therapy | 0 | 0.00 | 0 | 0.00 | 0 |
| 4 – Oxygen by mask or nasal prongs | 0 | 0.00 | 1 | 0.24 | 1 |
| 5 – Non-invasive ventilation or high-flow oxygen | 0 | 0.00 | 0 | 0.00 | 0 |
| 6 – Intubation and mechanical ventilation | 0 | 0.00 | 0 | 0.00 | 0 |
| 7 – Ventilation + additional organ support – pressors, RRT, ECMO | 0 | 0.00 | 0 | 0.00 | 0 |
| 8 – Death | 0 | 0.00 | 0 | 0.00 | 0 |
| Severe COVID-19 (last two weeks) | | | | | |
| Examination in an emergency department without hospitalization | 3 | 0.72 | 5 | 1.18 | 10 |
| Hospitalization | 4 | 0.96 | 3 | 0.71 | 7 |
| Hospitalization with a continuous oxygen requirement of ≥24 hours | 0 | 0.00 | 1 | 0.24 | 0 |
| Intensive care requirement | 0 | 0.00 | 0 | 0.00 | 0 |
| Ventilation requirement | 0 | 0.00 | 0 | 0.00 | 0 |
| Death by COVID-19 | 0 | 0.00 | 0 | 0.00 | 0 |
| Duration of hospitalization [days] | | | | | |
| 0 | 413 | 99.04 | 422 | 99.28 | 841 |
| 2 | 1 | 0.24 | 0 | 0.00 | 1 |
| 4 | 0 | 0.00 | 1 | 0.24 | 1 |
| 5 | 1 | 0.24 | 1 | 0.24 | 2 |
| 7 | 1 | 0.24 | 0 | 0.00 | 1 |
| 8 | 1 | 0.24 | 1 | 0.24 | 2 |

Supplementary Table 28: Severe COVID-19, Serious Adverse Events and emergency treatment in the safety population at week 4.

| Events / status at week 4 | Nicotinamide n=448 | | Placebo n=452 | | Total n=900 |
|---|-----------------------|--------|------------------|-------|----------------|
| | n | % | n | % | n |
| Missing | 36 | 8.04 | 34 | 7.52 | 70 |
| WHO Scale (current status at week 4 interview) | | | | | |
| 0 – No clinical or virological evidence of infection | 0 | 0.00 | 0 | 0.00 | 0 |
| 1 – No limitation of activities | 276 | 66.99 | 281 | 67.22 | 557 |
| 2 – Limitation of activities | 136 | 33.01 | 137 | 32.78 | 273 |
| 3 – Hospitalized, no oxygen therapy | 0 | 0.00 | 0 | 0.00 | 0 |
| 4 – Oxygen by mask or nasal prongs | 0 | 0.00 | 0 | 0.00 | 0 |
| 5 – Non-invasive ventilation or high-flow oxygen | 0 | 0.00 | 0 | 0.00 | 0 |
| 6 – Intubation and mechanical ventilation | 0 | 0.00 | 0 | 0.00 | 0 |
| 7 – Ventilation + additional organ support – pressors, RRT, ECMO | 0 | 0.00 | 0 | 0.00 | 0 |
| 8 – Death | 0 | 0.00 | 0 | 0.00 | 0 |
| Severe COVID-19 (last two weeks) | | | | | |
| Examination in an emergency department without hospitalization | 1 | 0.24 | 2 | 0.48 | 3 |
| Hospitalization | 0 | 0.00 | 1 | 0.24 | 1 |
| Hospitalization with a continuous oxygen requirement of ≥24 hours | 0 | 0.00 | 0 | 0.00 | 0 |
| Intensive care requirement | 0 | 0.00 | 0 | 0.00 | 0 |
| Ventilation requirement | 0 | 0.00 | 0 | 0.00 | 0 |
| Death by COVID-19 | 0 | 0.00 | 0 | 0.00 | 0 |
| Duration of hospitalization [days] | | | | | |
| 0 | 412 | 100.00 | 417 | 99.76 | 829 |
| 2 (plus 8 before Week 2 interview) | 0 | 0.00 | 1 | 0.24 | 1 |

Supplementary Table 29: Severe COVID-19, Serious Adverse Events and emergency treatment in the safety population at week 6.

| Events / status at week 6 | Nicotinamide n=448 | | Placebo n=452 | | Total n=900 |
|---|-----------------------|--------|------------------|--------|----------------|
| | n | % | n | % | n |
| missing | 46 | 10.27 | 34 | 7.52 | 80 |
| WHO Scale (current status at week 6 interview) | | | | | |
| 0 – No clinical or virological evidence of infection | 0 | 0.00 | 0 | 0.00 | 0 |
| 1 – No limitation of activities | 309 | 76.87 | 333 | 79.67 | 642 |
| 2 – Limitation of activities | 93 | 23.13 | 85 | 20.33 | 178 |
| 3 – Hospitalized, no oxygen therapy | 0 | 0.00 | 0 | 0.00 | 0 |
| 4 – Oxygen by mask or nasal prongs | 0 | 0.00 | 0 | 0.00 | 0 |
| 5 – Non-invasive ventilation or high-flow oxygen | 0 | 0.00 | 0 | 0.00 | 0 |
| 6 – Intubation and mechanical ventilation | 0 | 0.00 | 0 | 0.00 | 0 |
| 7 – Ventilation + additional organ support – pressors, RRT, ECMO | 0 | 0.00 | 0 | 0.00 | 0 |
| 8 – Death | 0 | 0.00 | 0 | 0.00 | 0 |
| Severe COVID-19 (last two weeks) | | | | | |
| Examination in an emergency department without hospitalization | 2 | 0.50 | 0 | 0.00 | 2 |
| Hospitalization | 0 | 0.00 | 0 | 0.00 | 0 |
| Hospitalization with a continuous oxygen requirement of ≥24 hours | 0 | 0.00 | 0 | 0.00 | 0 |
| Intensive care requirement | 0 | 0.00 | 0 | 0.00 | 0 |
| Ventilation requirement | 0 | 0.00 | 0 | 0.00 | 0 |
| Death by COVID-19 | 0 | 0.00 | 0 | 0.00 | 0 |
| Duration of hospitalization [days] | | | | | |
| 0 | 402 | 100.00 | 418 | 100.00 | 820 |

3. Supplementary information on methods for COVit-2

3.1. Trial procedures and design of COVit-2

The COVit-2 trial was performed remotely. Patients had to be able to give electronic written informed consent, which was explained and confirmed by a direct telephone call with the patient.

Patients were recruited through 24 independent diagnostic laboratory service providers with a total of 71 sites all over Germany. The following alphabetical list also contains the key collaborators:

1. alphaomega Labor GbR (PD Dr. Grit Ackermann, Sandra Wolf), Leipzig.
2. AniCon Labor GmbH (Dr. Klaus-Peter Behr), Höltinghausen.
3. Bioscientia Healthcare GmbH (Dr. Oliver Harzer), Ingelheim.
4. Centogene GmbH (Prof. Dr. Peter Bauer), Rostock.
5. Labopart – Labor Chemnitz (Dr. Michael Gerber), Chemnitz.
6. Laborärztliche Gemeinschaftspraxis Lübeck (Dr. Andreas Bobrowski), Lübeck.
7. Labor Becker & Kollegen MVZ GbR (Prof. Dr. Dr. Jürgen Durner), Munich.
8. Labor Blumenstraße (Dr. Frank Wietschel), Erfurt.
9. Labor Dr. Heidrich & Kollegen MVZ GmbH (Prof. Dr. Matthias Maaß), Hamburg.
10. Labor Dr. Krause & Kollegen MVZ GmbH (Dr. Thomas Lorentz, Dr. Olaf Grobe), Kiel.
11. Labor Dr. von Froreich GmbH (Prof. Dr. Tammo von Schrenck), Hamburg.
12. Labor Mohr (Harald Mohr), Kiel.
13. LADR Laboratory Group Dr. Kramer & Colleagues (Prof. Dr. Jan Kramer), Geesthacht.
14. Medizinisches Labor Ostsachsen MVZ GbR (Thomas Kirchner), Dresden.
15. Medizinisches Labor Westsachsen MVZ GbR (PD Dr. Dr. Peter Reichardt), Zwickau.
16. MVZ Dr. Eberhard & Partner Dortmund ÜBAG (Albert Pranada), Dortmund.
17. MVZ Dr. Stein + Kollegen (Dr. Dietmar Dreßen, Dr. Jelena Cucuz), Mönchengladbach.
18. MVZ Humangenetik Ulm GbR (PD Dr. Dietmar Plonné), Ulm.
19. MVZ Labor 28 GmbH (Dr. Michael Müller), Berlin.
20. MVZ Labor Dortmund (Dr. Matthias Aymanns), Dortmund.
21. MVZ Labor PD Dr. Volkmann und Kollegen GbR (PD Dr. Martin Volkmann), Karlsruhe.
22. MVZ Labor Ludwigsburg GbR (Prof. Dr. Rüdiger Braun), Ludwigsburg.
23. MVZ Medizinische Labore Dessau Kassel GmbH (Dr. Juliane Böttcher-Lorenz, Dr. Christina Kiel), Dessau-Roßlau.
24. Praxis für Labormedizin und Mikrobiologie ÜBAG GbR (Dr. Silke Biermann-Göcke), Bochum.

All patients who tested positive in the recruitment period at the laboratory sites were contacted together with the transmission of their laboratory results. Patients volunteered their identity to the recruitment center and after verification of key data including the referring laboratories and key inclusion criteria such as the test date, patients received the personalized patient information and a participation code, and could give informed consent on a secure trial website. Subsequently, they were contacted by telephone to check all inclusion and exclusion criteria and explain the informed consent. Immediately after confirming eligibility, patients were randomized and received the intervention and the optional paper questionnaires (SF-36 questionnaire: RAND 36-Item Health Survey 1.0; FACIT-F: 40-item Functional Assessment of Chronic Illness Therapy – Fatigue) by next-day courier shipment. Eligible patients were randomly assigned in a 1:1 ratio, by means of a randomization code in the trial database, to self-administer daily with breakfast either 1 g/d nicotinamide (500 mg immediate-release nicotinamide and 500 mg controlled-ileocolonic-release nicotinamide [CICR-NAM]) or matched placebo, for 4 weeks.

Nicotinamide is Generally Recognized As Safe (GRAS) by the FDA and has been assigned a daily Tolerable Upper Intake Level of 900 mg/d as a dietary supplement (vitamin B3) by the European authorities (EFSA).⁴ Immediate-release nicotinamide was purchased as 500-mg dietary supplement tablets (NicoPel®; Derma Enzinger; Ainring, Germany). Corresponding placebo tablets were purchased from FAGRON (Barsbüttel, Germany). The 500-mg CICR-NAM tablets and corresponding placebo tablets were manufactured by NextPharma (Göttingen, Germany). Tablets were released by the pharmacy of the University Hospital Schleswig-Holstein (Kiel, Germany). Together with the trial intervention, patients received administration instructions which were additionally explained to them in detail at the baseline visit.

Patients underwent structured telephone interviews conducted by blinded personnel at weeks 2, 4 and 6 as well as 6 months after baseline. Due to the patient-focused nature of the interview questions, patients usually did not have to retrieve data from their attending physician or other sources. Concomitant medication and supplements were recorded in detail at each interview. Any nicotinamide intake from dietary supplements was far below the intervention dose in the trial (1,000 mg/d). The interviewers queried symptoms one after another in a structured manner, according to SOPs and carefully instructed about not introducing bias or asking any leading questions. The interviewers made sure that the patients understood each question correctly. For example, it was made clear

that the reference point for performance drop or a reduced ability to perform normal activities (the two main and closely related endpoints pertaining to physical performance) was the health status of the patient directly prior to SARS-CoV-2 infection. Compliance was surveyed by tablet count during each interview and through specific questions. After week 6, patients returned the remaining trial supplements for accountability and destruction as well as the optional paper questionnaires completed on the same day of the interviews. Optional stool samples (at the time of each interview) and optional capillary blood samples for measuring levels of antibodies directed against the nucleocapsid (N) or spike (S) proteins of SARS-CoV-2 (after at least 6 months; AProof® Duo Test; Adversis Pharma/AP Diagnostics GmbH, Leipzig, Germany) were collected from patients all over Germany via mail. All trial procedures were documented and quality monitored, and all personnel received formal training.

3.2. Lists of COVID-19 symptoms queried by telephone interviews in COVit-2

Binary queries: is the symptom present or not?

A patient is usually fully aware of his/her normal physical performance and can clearly report any even minor discrepancies. This defines the binary primary endpoint “performance drop” as a both robust and sensitive means to detect any patient-reported physical impairment (see Discussion).

- Performance drop (**primary endpoint**)
- Fatigue (**3rd key secondary endpoint**)

- Fever
- Chills
- Shortness of breath
- Whistling/wheezing breathing
- Cough
- Cough with sputum production
- Rhinitis/rhinorrhea
- Sore throat
- Hoarseness
- Pneumonia

- Muscle pain
- Joint pain
- Limb pain
- Chest pain
- Headache

- Abdominal pain
- Diarrhea
- Nausea
- Vomiting
- Loss of appetite/lower food intake
- Other gastrointestinal symptoms (reported individually)

- Impaired sense of smell
- Impaired sense of taste

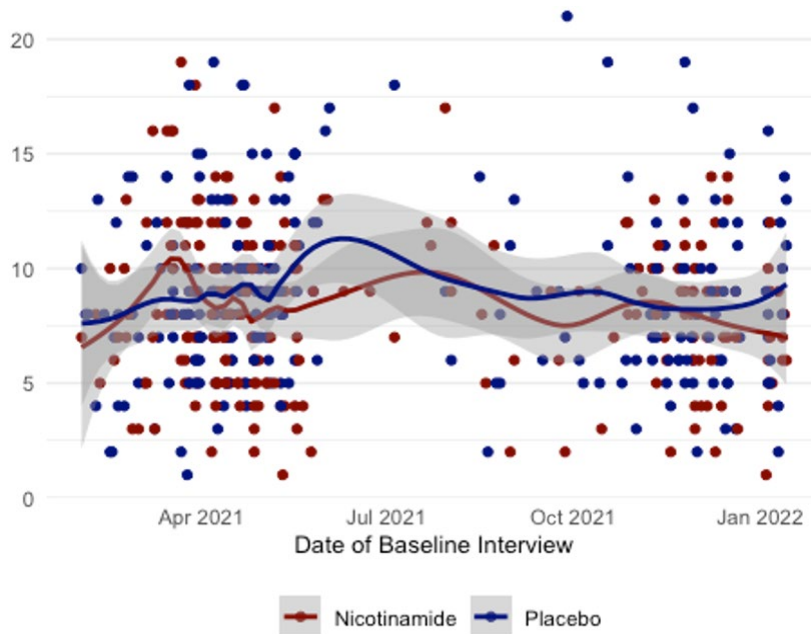
- Confusion
- Dizziness
- Conjunctivitis
- Skin rash
- Hair loss
- Other symptoms (reported individually)

Ordinal queries from a complaint scale for lower respiratory tract infections:

Gradations: 0 = normal, 1 = very minor problem, 2 = minor problem, 3 = moderately poor, 4 = poor, 5 = very poor, 6 = maximally poor.

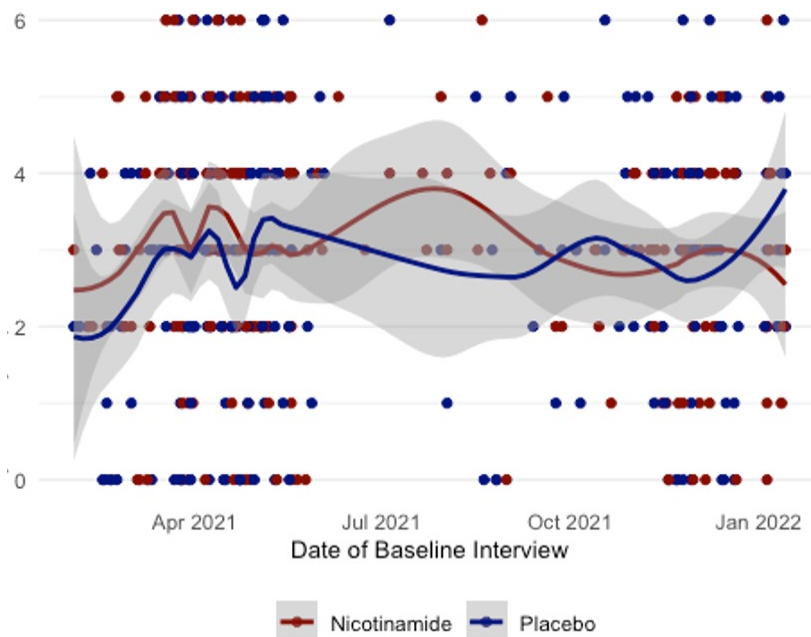
- Ability to perform normal activities (**1st key secondary endpoint**)
- Cough (**2nd key secondary endpoint**)
- Mucus production
- Shortness of breath
- Sleep
- General feeling of sickness

3.3. Symptoms and risk factors at baseline in COVit-2



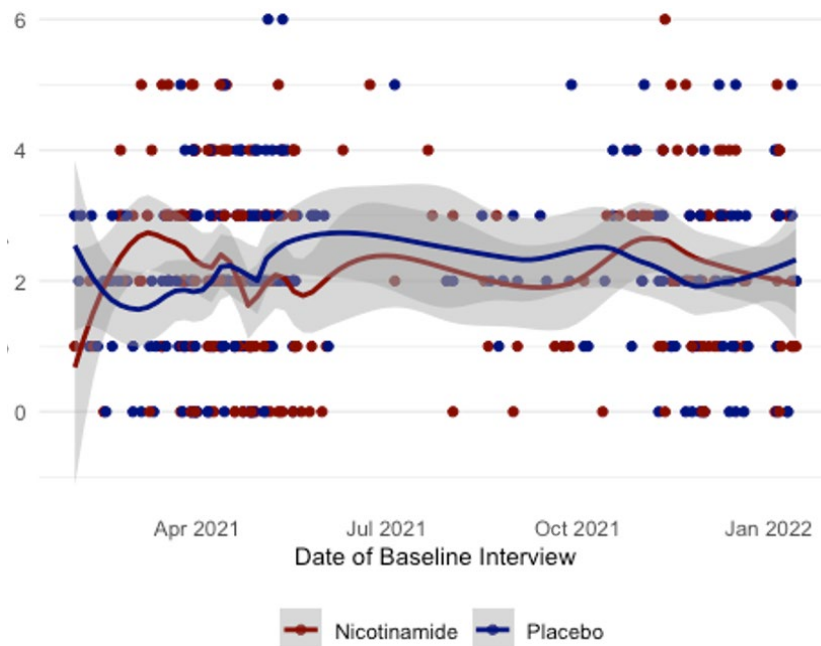
Supplementary Fig. 9: Number of symptoms reported at baseline in the RFITT population.

Each dot represents one patient (n=500). Lines were produced with locally weighted scatterplot (LOESS) smoothing in R. Grey shades are 95% CI bounds of LOESS smoothing.



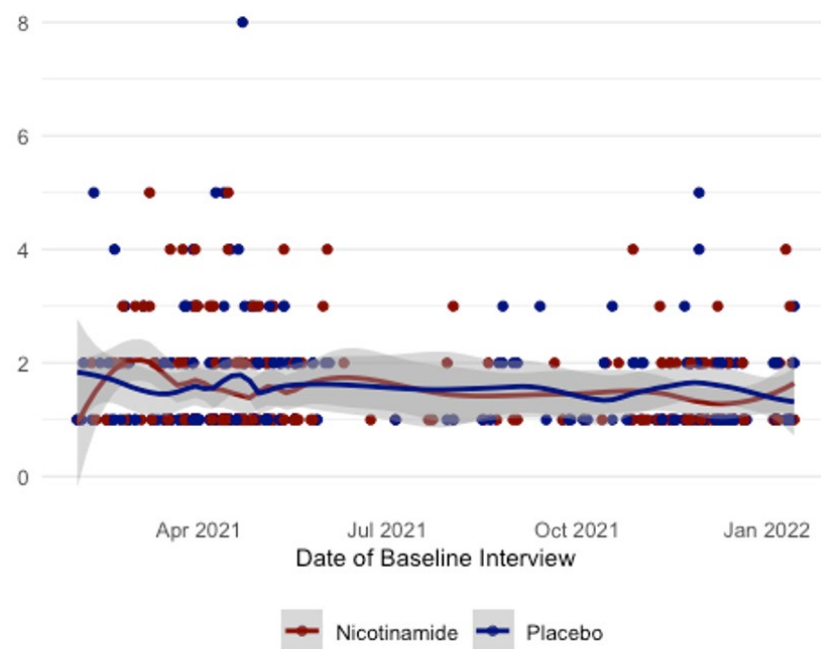
Supplementary Fig. 10: Intensity of reduced ability to perform normal activities reported at baseline in the RFITT population.

Each dot represents one patient (n=500). Lines were produced with locally weighted scatterplot (LOESS) smoothing in R. Grey shades are 95% CI bounds of LOESS smoothing.



Supplementary Fig. 11: Intensity of cough reported at baseline in the RFITT population.

Each dot represents one patient (n=500). Lines were produced with locally weighted scatterplot (LOESS) smoothing in R. Grey shades are 95% CI bounds of LOESS smoothing.



Supplementary Fig. 12: Number of risk factors for severe COVID-19 reported at baseline in the RFITT population.

Each dot represents one patient (n=500). Lines were produced with locally weighted scatterplot (LOESS) smoothing in R. Grey shades are 95% CI bounds of LOESS smoothing.

3.4. Analysis populations and risk factor subgroups in COVit-2

The safety population included all randomized subjects.

The intention-to-treat (ITT) population included all randomized subjects who received at least one dose of the intervention.

Populations and risk groups were defined in the statistical analysis plans (SAPs) for the analyses of acute COVID-19 and for the 6-month follow-up (see sections 6 and 7 of this Supplement, respectively). The RFITT (risk factor intention-to-treat) population was defined as the primary analysis population for efficacy for acute COVID-19. The RFITT population comprised all subjects of the ITT population with a least one characteristic or underlying medical condition associated with an increased risk of developing severe illness from COVID-19 (cf. <https://www.cdc.gov/covid/hcp/clinical-care/underlying-conditions.html>, last accessed on 11-Mar-2025), selected from the group of traits consisting of

- an age of ≥ 60 years;
- a body mass index of ≥ 30.0 ;
- type 1 diabetes;
- type 2 diabetes;
- cardiovascular diseases;
- high blood pressure;
- stroke;
- asthma;
- chronic obstructive pulmonary disease;
- other chronic lung diseases;
- current or former smokers (the latter being defined as patients who smoked more than 100 cigarettes or other smoking products in total so far, but have not smoked for at least 4 weeks);
- chronic liver diseases;
- chronic kidney diseases;
- cancer;
- organ transplants;
- current immunosuppressive therapy;
- chronic neurological diseases (multiple sclerosis, Parkinson's disease);

The per-protocol populations (PP and RFPP, respectively) included patients of ITT and RFITT, respectively, who did not

- drop out or
- comply regarding investigational product intake for at least 80%, i.e. intake of investigational product for at least 11 of 14 days between each study interval: week 0 – week 2 and week 2 – week 4.

The per-protocol populations were only approximately 5% smaller than the ITT populations ($n=831$ in PP vs. $n=867$ in ITT [95.8% per protocol] and $n=472$ in RFPP vs. $n=500$ in RFITT [94.4% per protocol], respectively).

The following pre-defined risk subgroups were investigated to differentiate their contribution to the primary and key secondary endpoint results for RFITT in acute COVID-19:

1. Age of ≥ 60 years.
2. Body mass index of ≥ 30.0 or type 2 diabetes.
3. Cardiovascular diseases, high blood pressure or stroke.
4. Asthma, chronic obstructive pulmonary disease or other chronic lung diseases.
5. Current or former smokers (the latter being defined as patients who smoked more than 100 cigarettes or other smoking products in total so far, but have not smoked for at least 4 weeks).

For the analysis of post-COVID syndrome (PCS) at the 6-month follow-up, the primary analysis population was the ITT population.

In order to identify patients at risk for developing PCS, baseline characteristics and symptoms of all available patients of the placebo population ($n=425$) were used to delineate predictors for a PCS score⁵ of ≥ 5 at month 6 with a significance threshold of $P < 0.05$. The following predictors were found:

- medical history: age > 60 years, chronic obstructive pulmonary disease, chronic inflammatory disease, depression, other chronic diseases, permanent intake of medication;
- symptoms from the onset of COVID-19 until the trial baseline: shortness of breath, whistling/wheezing breathing, absence of rhinitis/rhinorrhea, limb pain, nausea, dizziness;

- at baseline: shortness of breath, whistling/wheezing breathing, absence of rhinitis/rhinorrhea, conjunctivitis, chills, muscle pain, limb pain, dizziness, limitation of activity (a score of 2 on the WHO scale of COVID-19 severity developed in 2020).⁶

In order to be included into a subgroup of patients at risk for PCS, patients had to have more than 5 of these predictors at baseline.

A further subgroup was established based on improvement in the primary endpoint or one of the three key secondary endpoints (see section 3.2 of this Supplement) in the acute phase of the disease.

3.5. Methods for gut microbiome analyses in COVIT-2

Stool sample collection and selection of the 16S phylogenomic and metagenomics subcohorts

Native samples were collected and shipped by the participants using collection tubes in special return envelopes. Immediately upon arrival at the central laboratory at the Institute of Clinical Molecular Biology (Kiel University and University Hospital Schleswig-Holstein, Kiel, Germany), samples were frozen and stored at -80°C until DNA extraction. Only samples from complete sample series including week 0, week 2, week 4 and week 6 were used for the analyses.

Stool sampling was optional and conducted during periods of strict quarantine regulations and/or lockdowns. Only a subset of participants provided samples that met the quality criteria for analysis. The main quality control attributes for the selection of stool samples for analysis were the precise sequence of collection dates, the duration of shipping intervals and sufficient sample volumes.

A subcohort of 70 individuals was selected for an initial exploration of the microbiome using 16S rRNA amplicon sequencing. Additionally, a subcohort of 18 individuals was selected based on a targeted matching process. Patients were matched by age, sex, BMI, and key COVID-19-related symptoms (fever, cough, impaired sense of smell or impaired sense of taste at baseline), resulting in the selection of 9 patients from the nicotinamide group and 9 from the placebo group, independent of the initial cohort. Statistical tests confirmed no significant baseline differences in demographics between the nicotinamide and placebo subgroups selected for 16S phylogenome and metagenome analyses (Supplementary Tables 16 and 17).

16S rRNA sequencing and preprocessing

Fecal DNA from 280 stool samples was extracted using the DNeasy PowerSoil Pro Kit (Qiagen) following the manufacturer's protocol. Extracted DNA was eluted from the spin filter silica membrane with 100 μl of elution buffer and stored at -80°C . 16S profiling and MiSeq sequencing was performed as described earlier⁷ with the following modifications: the V3-V4 region of the 16S gene was amplified using the dual barcoded primers 341F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT). Each primer contained additional sequences for a 12 base Golay barcode, Illumina adaptor and a linker sequence. PCR was performed using the Phusion Hot Start Flex 2X Master Mix (New England Biolabs, Frankfurt am Main, Germany) in a GeneAmp PCR system 9700 (Applied Biosystems/Thermo Fisher Scientific, Darmstadt, Germany) and the following program: 98°C for 3 min, 25x (98°C for 20 s, 55°C for 30 s, 72°C for 45 s), 72°C for 10 min, hold at 4°C . Performance of the PCR reactions was checked using agarose gel electrophoresis. Normalization was performed using the SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, Darmstadt, Germany) following the manufacturer's instructions. Equal volumes of SequalPrep-normalized amplicons were pooled and the pool then handed to the Competence Centre for Genomic Analysis (Kiel, Germany) for sequencing on an Illumina MiSeq v3.0 (2 x 300 nt).

To characterize the microbiome composition, we first used the QIIME2 environment⁸ (release 2021.4) for processing the reads, which are deposited and available at ENA under the accession code PRJEB61276 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB61276>; last accessed on 11-Mar-2025). The paired-end sequences were filtered, denoised, merged and chimera-removed using the DADA2 plugin⁹ (version 2021.2) implemented in QIIME2. After this step, the 16S rRNA gene amplicon sequencing reads retained on average 10,566 reads per sample. Where applicable, 16S rRNA gene sequence data were rarefied to a uniform sequencing depth of 5,000 reads, ensuring consistent coverage across samples for downstream analyses. In these downstream analyses, only samples with at least 5,000 reads were retained. Samples that did not meet this minimum threshold criterion were excluded.

To assess taxonomic assignments, we employed the QIIME2 scikit-learn classifier trained on the SILVA¹⁰ (release 138) reference database (<https://zenodo.org/records/6395539>; last accessed on 11-Mar-2025).

Metagenome data processing

Shotgun metagenomics was performed at the Competence Centre for Genomic Analysis (Kiel, Germany). Metagenomes were processed using TOFU-MAaPO v1.2.2 (<https://github.com/ikmb/TOFU-MAaPO>; last accessed on 11-Mar-2025) that relies on the bioBakery 3 environment² (MetaPhlAn 3.0 and HUMAnN 3.0). Raw reads were quality-trimmed and mapped to the human genome (hg19) to discern between sequenced reads from the host (human) and the gut microbiome bacteria. Only samples containing >1 Gbp after trimming and host decontamination were kept in the analysis.

Taxonomic and functional potential analysis

The metagenomics analysis was performed using the general guidelines.¹¹ Where applicable, metagenomic sequence data were rarefied to a uniform sequencing depth of 1,000,000 reads, ensuring consistent coverage across

samples for downstream analyses. Taxonomic features and quantification of microbial communities' relative abundances on 72 samples were done by using MetaPhlAn 3.0^{ref. 2} with default parameters. MetaPhlAn 3.0 relies on a database of clade-specific marker genes identified from ~13,500 bacterial/archaeal and ~3,500 viral genomes. Likewise, functional potential profiling (stratified pathways, gene families and enzyme categories) in the same samples was performed using HUMAnN 3.0,^{ref. 2} with default parameters. Functional potential analysis was based on MetaCyc v26.1 (<https://metacyc.org/>; last accessed on 11-Mar-2025).¹² All abundances were transformed to CPMs before statistical analysis.

Public cohort data

To further investigate if nicotinamide mitigates COVID-19-associated gut microbiota changes, we decided to explore the functional potential of stool samples of a longitudinal public cohort of COVID-19 individuals who were hospitalized in the Charité Universitätsmedizin Berlin.¹³ We analyzed the longitudinal gut microbiota data of 15 healthy individuals, 19 patients with mild COVID-19 and 7 patients with severe COVID-19, from a total of 81 stool samples. All the fastq files were processed exactly as described above for our metagenomics samples.

Statistics

Most of the univariate and multivariate analyses were done in the R statistical software¹⁴ (v.4.2.1) under phyloseq¹⁵ (v.1.40.0), vegan¹⁶ (v.2.6-2) and MAASLin2^{ref. 17} (v.1.10.0).

Within-sample diversity (α -diversity) was explored by computing different diversity indexes (Chao1, Shannon, Inverse Simpson, Fisher, Observed Amplicon Sequence Variants [ASVs]) on ASV abundance data and finding differences among certain groups (Wilcoxon signed rank test or Kruskal-Wallis tests were performed).

For cross-sectional comparisons of microbial diversity between intervention groups, we used the two-sided Wilcoxon rank-sum test.

To assess the effect of time of microbial diversity, we fitted linear mixed-effects models using the lme4 package in R (v1.1-34). The response variable was modeled as a function of time, with subjects as random intercepts to account for repeated measures. Furthermore, a likelihood ratio test was used to compare the full model (Diversity index ~ time + (1|Subject)) with a null model (Diversity index ~ 1 + (1|Subject)). Significance was determined based on the chi-square statistic (χ^2) and associated P value.

Between-sample diversity (β -diversity) and the differences between intervention groups were explored and visualized in a distance-based redundancy analysis (db-RDA) plot. Quantification was performed by analyzing Aitchinson distances on centered log ratios from ASVs abundance data. Associations of microbiome composition to external covariates were tested with the implementation of PERMANOVA models (using adonis2 function from the vegan package). The P and R² values were determined by 10,000 permutations using Intervention Group, Age, and key COVID-19-related symptoms as covariables in the model.

To detect differences in changes of microbial features, taxonomic (16S rRNA) or pathways (shotgun sequencing) between the intervention groups over time, we built linear mixed models in the MaAslin2 package¹⁷ where we adjusted for BMI and sex as fixed effect variables, and individual and age group as random effect variables. The final model used for this analysis is as follows: Feature Abundances ~ Intervention + Time + BMI + Sex + (1|Individual) + (1|Age Group). P values were corrected for multiple hypothesis testing using the Benjamini-Hochberg procedure, and a false discovery rate (FDR) <0.25 was defined as the significant threshold.

To detect differences in changes of pathways in the public dataset from Essex et al.,¹³ we built a similar linear mixed model in which we included BMI, sex, WHO ordinal scale of clinical improvement (OSCI) class, visits and antibiotic usage at sampling day as fixed effect variables and individual and age group as random effect variables. The final model used for this analysis is as follows: Feature Abundances ~ OSCI Class + Visit + BMI + Sex + AbxSamp + (1|Individual) + (1|Age Group). We utilized this model to identify differentially abundant pathways between OSCI classes (Healthy vs. Mild, Healthy vs. Severe) both at the onset of and during the hospitalization period in COVID-19 patients. The pathways identified as differentially abundant between OSCI classes in the Essex et al.¹³ cohort were subsequently compared to those observed between nicotinamide and placebo groups in our cohort.

To understand the major sources of variation in our microbiome data at the genus level, we applied variancePartition¹⁸ v.1.26.0, which uses linear mixed models to compute the attributable percentage of variation of a feature based on selected covariates (individual, age, body mass index, fever prior to baseline, nicotinamide intervention, COVID-19-specific symptoms).

3.6. Sample size and futility analysis in COVit-2

Data available from the literature available at the beginning of the COVit-2 trial and results of the COVit-1 pilot trial (see section 1 of this Supplement) suggested that 40% of symptomatic patients with COVID-19 become symptom-free during the spontaneous course of the disease within two weeks. An increase of the event rate of becoming symptom-free to 50% (relative risk 1.25) through the use of nicotinamide at a 5% level of significance and 80% power resulted in a required sample size of at least 385 patients per group (for details, see section 6 of this Supplement). With an assumed drop-out rate of 10%, 420 patients per group (assigned in a 1:1 ratio) were to be included. Based on the results of the futility analysis after 400 patients, the Data Management Board (DMB) of the COVit-2 trial recommended (1) to keep the previously planned sample size of approximately 840 patients and (2) to base the efficacy analysis on patients with at least one risk factor for developing severe COVID-19 (RFITT, see section 3.4 of this Supplement). To ensure that the subpopulation recruited after the futility analysis also included an adequate proportion of such patients, the frequencies of risk factors were monitored in a blinded manner and 900 patients were recruited as a safeguard against possibly increased dropout rates. The Principal Investigator and the blinded trial team members remained blinded until the end of the trial and received only the recommendation of the DMB.

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5. Trial protocols

This section contains

- the English translation of the protocol of the COVit-2 trial (18 pp.) and
- the original German trial protocol of the COVit-2 trial (19 pp.)

in the final version 2.3 of 13 December 2021

and

- the English translation of the protocol of the COVit-1 trial (12 pp.) resulting in the pilot study and
- the original German trial protocol of the COVit-1 trial (13 pp.)

in the final version 1.1 of 26 March 2020.

CLINICAL TRIAL PROTOCOL

Improvement of the Nutritional Status Regarding Nicotinamide (Vitamin B3) and the Disease Course of COVID-19

Trial Code:
COVit-2

Version: 2.3

Date: 13 December 2021

PI: Prof. Dr. Stefan Schreiber
Department of Internal Medicine I
University Medical Center Schleswig-Holstein, Campus Kiel
Arnold-Heller-Strasse 3, Building K1
24105 Kiel
Germany

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 - 19.9. Additional Informed Consent for the Antibody Test

1 Summary of the Project

Based on the literature, it seems likely that a nutritional intervention with nicotinamide (a form of vitamin B3) can support the therapy of SARS-CoV-2 infection (COVID-19) by, *e.g.*, improving the availability of tryptophan and its metabolism-supporting metabolites (*e.g.* NAD). A pilot phase of the COVit trial (COVit-1) surprisingly showed a significant effect of nicotinamide on the time to complete resolution of COVID-19 symptoms. In addition, diarrhea is a common symptom of COVID-19. Therefore, in a second part of the trial, 420 symptomatic patients each with confirmed SARS-CoV-2 infection shall self-administer 1,000 mg nicotinamide (500 mg conventional nicotinamide and 500 mg nicotinamide released in a controlled manner in the intestine) or corresponding placebos per day in a blinded fashion for 4 weeks. The primary endpoint of the trial is the occurrence of individual COVID-19 symptoms at week 0, week 2 (primary analysis time point), week 4 and week 6, and after 6 months. Secondary endpoints are the severity of COVID-19 symptoms, the occurrence and severity of symptoms at the 6-month follow-up (post-COVID-19 syndrome, PCS), the levels of antibodies against N-protein and S-protein of SARS-CoV-2 after at least 6 months, the complete resolution of symptoms at 2, 4 and 6 weeks, and the time from diagnosis to resolution of individual or all symptoms. Exploratory endpoints will include the WHO clinical scale for COVID-19 and any development of severe COVID-19 (emergency department examination, hospitalization with at least 24 hours of oxygen requirement, intensive care requirement, ventilator requirement, or death), changes in fatigue and quality of life, and various biomarkers. Patients are recruited after positive testing, receive the information for informed consent and can declare their participation via a website. After randomized distribution of the trial medication, the patients will be asked about their disease course in telephone interviews at baseline (week 0) and after 2, 4 and 6 weeks. Stool samples will be collected from up to 400 patients at week 0, week 2, week 4, week 6 and after 6 months. In addition, in up to 20 selected patients, various inflammatory markers and the metabolome, in particular tryptophan metabolism, are examined in addition to the blood count and standard blood profile. In these patients, the viral strain is also determined by sequencing from nasopharyngeal swabs. In selected patients, in addition to the blood parameters mentioned above, the pharmacokinetics of nicotinamide, nicotinic acid and nicotinuric acid as well as metabolites of nicotinamide and tryptophan metabolism will be examined in the first 48 hours after a single administration of the trial interventions. In the stool, the changes in microbiome (in 100–300 patients) as well as metagenome and metabolome (in a subgroup) will be analyzed. The trial aims to generate rapid results on whether nicotinamide supplementation can alleviate the disease course of COVID-19. In addition, a follow-up interview, a smell test, a cognition test validated for telephone interviews (T3MS) and a test for SARS-CoV-2 antibodies after at least 6 months will be used to investigate whether such supplementation has an impact on the PCS as well as the immune response and, if applicable, vaccination against SARS-CoV-2.

2 Responsibilities

Principal Investigator of the trial:

Prof. Dr. med. Stefan Schreiber

Department of Internal Medicine I

University Medical Center Schleswig-Holstein (UKSH), Campus Kiel

Arnold-Heller-Strasse 3, Building K1, 24105 Kiel, Germany

Tel.: +49-431-500-15101, e-mail: s.schreiber@mucosa.de

Responsible centers and contacts:

Trial centers:

Department of Internal Medicine I, UKSH, Campus Kiel.

For recruitment, a large number of medical practices, general hospitals or testing sites/laboratories in Germany that treat COVID-19 patients will be used.

Participating trial laboratories:

Institute of Clinical Chemistry, UKSH, Campus Kiel: blood analyses.

Fraunhofer ITEM (Hannover, Germany), MVZ Dr. Eberhard & Partner (Dortmund, Germany) and the Medical Center of the University of Munich (Germany): blood analyses.

Institute of Clinical Molecular Biology, Kiel University and UKSH, Campus Kiel: microbiome, metagenome and metabolome analyses in stool; blood analyses, SARS-CoV-2 sequencing. Adversis Pharma GmbH (Leipzig, Germany): antibody analyses in dried blood spot samples.

Telephone interviews:

Competence Network Intestinal Diseases e.V., Kiel (Germany).

Data management and statistics:

Competence Network Intestinal Diseases e.V., Kiel (Germany): data management and statistics.

Novustat AG, Wollerau (Switzerland): external statistics (particularly futility analysis).

Biobanking and sample management:

Institute of Clinical Molecular Biology, Kiel University and UKSH, Campus Kiel.

Funding:

The trial is funded by the Department of Internal Medicine I (UKSH, Campus Kiel), by start-up funding from the state of Schleswig-Holstein (project DOI30) and by the Cluster of Excellence PMI. The trial protocol was registered in the German Register of Clinical Trials (DRKS) prior to the start of recruitment and will be updated accordingly following a positive assessment. In parallel, the trial is registered at ClinicalTrials.gov.

3 Scientific Background

The availability of tryptophan and its metabolites, especially nicotinamide (a form of vitamin B3) as a building block of NAD and NADP, are important factors in chronic systemic inflammation. Here, an evolutionarily ancient defense mechanism can be misdirected by the body restricting energy use in cells analogous to iron reduction during inflammation (Boergeling & Ludwig 2017, FEBS J. 284:218). While this makes sense in simple organisms, such mechanisms lead to dysfunctions (e.g. inflammation-induced anemia) in complex organisms. Reduced levels of nicotinamide and thus NAD(P) compromise the immune defense against coronaviruses (Heer et al. 2020, J. Biol. Chem. 295:17986) and cell metabolism, especially of macrophages and epithelial cells. The latter in particular are subject to a critically high rate of renewal in the gut and lungs. In addition, kynurenine, as the main degradation product of tryptophan, can also have a strong immune-activating effect. Another link between SARS-CoV-2 beyond inflammation and the immune system could also be the SARS-CoV-2 entry receptor ACE2, as the presence of ACE2 on the cell surface allows uptake of tryptophan via the receptor B0AT1. In severe courses of influenza, as in chronic inflammation, increased tryptophan degradation and thus increased kynurenine levels are observed, and inhibition of tryptophan degradation has beneficial effects in animal models (Pizzini et al. 2019, Influenza Other Respir. Viruses 13:603; Boergeling & Ludwig 2017, FEBS J. 284:218). In turn, there is evidence in a wide variety of virus types that nicotinamide can reduce viral replication and support the body's defense mechanisms, e.g. in vaccinia (Child et al. 1988, Virus Res. 9:119), HIV (Murray 2003, Clin. Infect. Dis. 36:453), enteroviruses (Moell et al. 2009, J. Med. Virol. 81:1082) or hepatitis B (Li et al. 2016, Arch. Virol. 161:621). Sufficient supply of B vitamins and particularly nicotinamide to strengthen the immune system is also recommended to combat SARS-CoV-2 infection (Zhang & Liu 2020, J. Med. Virol. 92:479; Gharote 2020, Ind. J. Med. Sci. 72:25; Shakoore et al. 2021, Maturitas 144:108). Here, the improvement of the defense against secondary bacterial infections in disease models is particularly emphasized (Zhang & Liu 2020, J. Med. Virol. 92:479). The reduced oxygen saturation observed in one study at a dose of 400 mg/kg (this would correspond to an arguably toxic dose of 28 g in a 70-kg person) has no significance for a dietary intervention with 1,000 mg total dose and given the acceptable daily intake of 900 mg/day (EFSA Panel on Dietetic Products, Nutrition and Allergies 2014, EFSA J. 12:3759). In the COVit trial, 1,000 mg are administered, which is far below potentially harmful doses of several grams per day and very close to the ADI [OECD-SIDS: 3-pyridinecarboxamide (nicotinamide), SIDS Initial Assessment Report for SIAM 15, Boston, Massachusetts, 22-25 October 2002]. Meanwhile, several publications suggest the use of nicotinamide in COVID-19, but there are no trial data available (Shi et al. 2020, Cell Death Differ. 27:1451; Gharote 2020, Ind. J. Med. Sci. 72:25; Mehmehl et al. 2020, Nutrients 12:1616; Shakoore et al. 2021, Maturitas 144:108; Heer et al. 2020, J. Biol. Chem. 295:17986).

In the pilot phase of the COVit trial (COVit-1), at first n=8 patients each received 1,000 mg of conventional nicotinamide or 245 mg of silica as placebo. During a quality control of the data collection, it was surprisingly observed that 4 of 8 patients (50%) in the nicotinamide group were already completely symptom-free after two weeks of administration, whereas this was the case in only one of the 8 patients in the control group. The evaluation of the COVit pilot trial with n=28 per group showed a significant reduction of the time to complete resolution of symptoms, which was driven by effects in female patients. From the literature, depending on the patient population, very long convalescences were also to be expected, e.g.

- 43% of patients were symptom-free at 14-21 days after positive SARS-CoV-2 test (Tenforde et al. 2020, MMWR 69:99),

- <30% without respiratory symptoms after 1 month (Marshall 2020, Nature 585:339),
- 32% symptom-free at 30 days and still only 34% at 60 days after SARS-CoV-2 diagnosis (Carvalho-Schneider et al. 2021, Clin. Microbiol. Infect. 27:258), or
- only 12.6% symptom-free after a mean of 60 days (Carfi et al. 2020, JAMA 324:604).

Moreover, since the start of the originally planned COVit trial, COVID-19 has been shown to be a systemic disease with an unexpectedly high frequency of gastrointestinal symptoms (Mitsuyama et al. 2020, J. Clin. Med. 9:3630). Recently, it has been published that the gut microbiome is also significantly and, in some cases, negatively affected by COVID-19 (Yeoh et al. 2021, Gut 70:698). Therefore, in the main phase of the trial (COVit-2), two different tablets, each containing 500 mg nicotinamide, will now be used as investigational drugs: the conventional immediate-release nicotinamide tablets from the pilot phase and the UKSH proprietary development CICR-NAM (controlled-ileocolonic-release nicotinamide), whose tablets release nicotinamide in a delayed and continuous manner starting in the lower small intestine. This is expected to increase the gastrointestinal beneficial effects of nicotinamide, specifically also on the microbiome and its interaction with the gut, as conventional nicotinamide is very rapidly absorbed into the circulation (Fangmann et al. 2018, Diabetes Care 41:398). Previous experiments with a CICR-NAM prototype have shown that systemic exposure from the new dosage form will be lower than in the pilot phase of the study (Fangmann et al. 2018, Diabetes Care 41:398), in which exposure was already close to the ADI anyway (see above). This will further reduce the already minimal risk of side effects.

The scientific background has evolved during the course of the study and has led to an adjustment of endpoints and analyses as part of the planned futility analysis and sample size review. This is explained in detail in Section 11.

4 Project Objectives

4.1 Primary Objective and Hypothesis

The primary objective of the trial is to investigate the hypothesis that COVID-19 patients lose individual COVID-19 symptoms more rapidly under real-world conditions if they supplement 1,000 mg of nicotinamide (500 mg of conventional nicotinamide and 500 mg of CICR-NAM).

4.2 Secondary Objectives

In addition to the primary objective mentioned above, the secondary objectives of the trial are to investigate the following parameters in more detail: severity of individual symptoms, occurrence and severity of symptoms at the 6-month follow-up (PCS), levels of antibodies against the N-protein and S-protein of SARS-CoV-2 after at least 6 months, complete resolution of symptoms at 2, 4, and 6 weeks, and time from diagnosis to freedom from individual or all symptoms.

4.3 Exploratory Objectives and Questions

The WHO clinical scale for COVID-19 and a development of severe COVID-19 (examination in an emergency department, hospitalization with at least 24 hours of oxygen requirement, intensive care requirement, ventilator requirement, or death) will be investigated in an exploratory fashion. Changes in fatigue and quality of life will be assessed using validated questionnaires (FACIT-F and SF-36). Further exploratory analyses in up to 20 selected patients will provide information on blood count, standard blood profile, various inflammatory

markers and the metabolome in the blood, in particular tryptophan metabolism. In these patients, the viral strain will also be determined by sequencing from nasopharyngeal swabs. In selected patients, detailed pharmacokinetics may follow. In the stool, changes in the microbiome (in 100–300 patients) as well as in the metagenome and metabolome (in a subgroup) will be analyzed.

5 Endpoints

The disease course of the nicotinamide-supplemented group and the placebo group will be compared using the following endpoints:

Primary endpoint:

Frequencies of individual COVID-19 symptoms at week 0, week 2 (primary time of analysis), week 4 and week 6 as well as after 6 months.

Secondary endpoints:

1. Severity of individual COVID-19 symptoms at week 0, week 2 (primary time of analysis), week 4 and week 6 as well as after 6 months.
2. As part of the 6-month follow-up: frequency and severity of symptoms characteristic of post-COVID-19 syndrome.

For this purpose, a validated smell test (Smell Identification Test™; Sensonics / MediSense) is performed by the patient. Furthermore, in addition to the FACIT-F and SF-36 questionnaires (as during the actual study period from week 0 to week 6), additional questionnaires (see Appendix 19.6.) will be completed on olfactory and gustatory abilities (incl. Questionnaire of Olfactory Disorders, QOD), on respiration (Multidimensional Dyspnea Profile, MDP), on mental state (Patient Health Questionnaire Depression, PHQ-8; Generalized Anxiety Disorder 7, GAD-7; Perceived Stress Scale, PSS; Brief Resilience Scale, BRS), on sleep quality (Pittsburgh Sleep Quality Index, PSQI), and on fatigue (Multidimensional Fatigue Inventory, MFI). In addition, a cognition test validated for telephone interviews (T3MS) will be performed.

3. Levels of antibodies directed against the N protein or S protein of SARS-CoV-2 after at least 6 months (anti-S protein stratified for presence and type of booster vaccination).
4. Complete symptom resolution after 2 weeks.
5. Complete symptom resolution after 4 weeks.
6. Complete symptom resolution after 6 weeks.
7. Time from diagnosis to resolution of individual symptoms (in days, up to 6 weeks).
8. Time from diagnosis to complete symptom resolution (in days, up to 6 weeks).

The following symptoms are queried in telephone interviews and explored individually in their course: Fatigue / tiredness / exhaustion / lack of strength, drop in performance / limited physical performance, fever (up to 39 °C or above), chills, shortness of breath, whistling or wheezing breathing, pneumonia, cough (with or without sputum production), rhinitis / runny nose / nasal mucus production / rhinorrhea, sore throat / pharyngitis or scratchy throat, hoarseness, muscle pain (myalgia), joint pain (arthralgia), limb pain, chest pain, headache,

abdominal pain, diarrhea, nausea, vomiting, loss of appetite / decreased food intake, other gastrointestinal symptoms (to be named individually by the patient), impaired sense of smell, impaired sense of taste, impaired consciousness / confusion, dizziness, conjunctivitis, skin rash, hair loss, and other symptoms (to be named individually by the patient).

In a subgroup of patients, daily changes in the severity of individual symptoms during the first 4 weeks are investigated in an exploratory fashion; gradations of symptoms: 0 = not at all, 1 = mild, 2 = moderate, 3 = severe, 4 = intolerable.

In addition, a complaint scale for lower respiratory tract infections is queried with the aspects of cough, mucus production, shortness of breath, sleep, ability to perform normal activities, and general feeling of illness with the following gradations: 0 = normal, 1 = very minor problem, 2 = minor problem, 3 = moderately poor, 4 = poor, 5 = very poor, 6 = maximally poor.

Exploratory endpoints:

1. World Health Organization (WHO) COVID-19 Ordinal Scale for Clinical Improvement (see <https://www.who.int/publications/i/item/covid-19-therapeutic-trial-synopsis>) at week 0 (baseline), week 2, week 4 and week 6 as well as after 6 months.
2. Occurrence of severe COVID-19, defined as achieving one of the following characteristics:
 - a. examination in an emergency department;
 - b. hospitalization with continuous oxygen requirement of at least 24 hours;
 - c. intensive care requirement;
 - d. ventilation requirement;
 - e. death by COVID-19.
3. Comparison between findings from the pilot phase of the trial and the second part of the trial.
4. Changes in fatigue (FACIT-F questionnaire).
5. Changes in quality of life (SF-36 questionnaire).
6. Changes in blood levels of tryptophan (in selected patients).
7. Changes in blood levels of tryptophan metabolites (in selected patients).
8. Changes in blood levels of inflammatory markers (C-reactive protein, interleukin-6, ferritin, neopterin, D-dimers) (in selected patients).
9. Changes in blood count and standard blood profile (in selected patients).
10. Changes in blood metabolome composition (in selected patients).
11. Strain of SARS-CoV-2 virus (in selected patients).
12. Changes in stool microbiome composition (in selected patients).
13. Changes in stool metagenome composition (in selected patients).
14. Changes in stool metabolome composition (in selected patients).
15. Pharmacokinetics (in selected patients).

6 Trial Design

This is a monocentric, randomized trial in which symptomatic COVID-19 patients receive dietary supplementation with 1,000 mg nicotinamide per day in tablet form (1 x 500 mg NicoPel, IFC Germany/Derma Enzinger, and 1 x 500 mg CICR-NAM, NextPharma/UKSH) or matching placebo tablets (Fagron and NextPharma/UKSH, respectively). In the pilot phase, the nicotinamide group received 2 x 500 mg NicoPel and the control group received 245 mg silica in a capsule (1 x 245 mg silica capsule, Twardy/Saluspharma). The less than perfect pairing of verum and placebo-like control was justified by the market availability of the investigational products and the tremendous urgency of the trial. It is assumed that the comparator group “remedy silica” had an effect size comparable to the placebo treatment, since silica consists mainly of inert silicon dioxide. However, as positive statements on the nutritional benefit have been established for silica as advertizable evaluations, the placebo was optimized for the second part of the trial. Double blinding was and is ensured as far as possible. For the patients, it is not clear from the packaging and intake instructions of the dietary supplement sent to them which investigational product they received. Data collection personnel on the telephones are instructed by SOP not to discuss the type of investigational product and are blinded as to the group allocation of the trial participants they are calling. All patients receive the usual clinical standard of care, and the nutritional supplement is purely additive to the standard of care.

7 Trial Population

The trial population to be investigated, totaling up to approximately 840 trial participants, involves patients with confirmed SARS-CoV-2 infection and symptoms, e.g. in the respiratory and/or gastrointestinal tract.

Inclusion criteria are:

- SARS-CoV-2 infection confirmed by laboratory findings; the positive test must not date back more than 7 days.
- Relevant infection symptoms, e.g. in the respiratory or gastrointestinal tract.
- The patient has been able to give written consent via a website before any trial procedure is performed and can comply with the trial-dependent prerequisites and requirements.
- The patient is of age (at least 18 years).

Exclusion criteria are:

- Current participation in another trial.
- Pregnancy or breastfeeding.
- Vaccination against SARS-CoV-2.

Recruitment will take place in Kiel, Germany, and in other physician offices, hospital centers, and testing sites/laboratories for SARS-CoV-2 in Germany.

8 Trial Procedures

8.1 Informed Consent

Treatment decisions for COVID-19 are outside the trial protocol, i.e. the indication is independent and established without influence by the research program. Only after the indication has been established, a decision is made regarding possible recruitment.

All patients in the respective centers (outpatient and inpatient) who meet all inclusion and exclusion criteria will be asked to participate in the trial. There will be no trial-specific procedure outside the clinical standard in screening. Patients will receive a written patient information with informed consent. If necessary, a hotline is available for queries and a medical consultation for informed consent. After sufficient time for consideration, the patient can then consent online and document his or her consent by printing out the relevant page for him or herself (subject consent). This procedure also allows patients in quarantine at home to participate. Only after online consent, the first telephone contact is made for data collection for week 0 (baseline), wherein at the beginning the patient's ability to consent is checked again by study staff on the telephone according to an SOP. Again, the patient can ask further questions and the medical hotline can be consulted.

Supplemental written informed consent will be obtained from patients for the olfactory test, the additional questionnaires, the cognition test, and the test for SARS-CoV-2 antibodies at the 6-month follow-up, as well as for the pharmacokinetic measurements.

8.2 Measures (Intervention/Control) and Target Sizes

After consent and fulfillment of all requirements, patients are randomly assigned to the nicotinamide arm or the placebo arm. The patient will be informed about this.

After randomization in a 1:1 ratio, control/interview calls are planned at week 0 (baseline) as well as week 2, week 4 and week 6. After approximately 6 months, another follow-up call is planned to clarify long-term symptoms. Together with the tablets, patients will receive printed questionnaires to characterize fatigue (FACIT-F) and quality of life (SF-36), which they should fill out themselves and return by prepaid envelope. In this context, wearables analogous to the A101/20 may also be used in perspective; this would then be specified and presented in more detail. Up to 400 patients will receive sample containers for stool samples, which will be sent to the IKMB via return envelope. The aim is to obtain 100–300 sample series for stool microbiome analyses, of which a representative portion will be additionally analyzed for changes in the stool metagenome and stool metabolome. In an expected number of up to 20 patients from Kiel and the surrounding area (up to 10 patients each with verum or placebo), additional blood samples will be taken at the interview time points and at week 1 to perform the exploratory blood analyses (see endpoints). In these patients, the viral strain will also be determined by sequencing from nasopharyngeal swabs at baseline. In up to 5 selected patients from this group, the pharmacokinetics of nicotinamide, nicotinic acid and nicotinuric acid, as well as of metabolites of nicotinamide and tryptophan metabolism, will also be assessed at a later time point during the first 48 hours after a single administration of the trial products.

Furthermore, a follow-up interview, additional questionnaires, a smell test, a cognition test and a test for SARS-CoV-2 antibodies after 6 months will be used to investigate whether supplementation with nicotinamide has an influence on the PCS as well as the immune response and possibly vaccination against SARS-CoV-2. The information obtained here is consistent (except for the additional antibody test) with the data collected in the COVIDOM trial and will allow for subsequent cross-linking of the two trials to be reviewed separately.

The telephone interviews are conducted by the Competence Network Intestinal Diseases e.V. For medical questions, a physician from the Department of Internal Medicine I calls back. The following parameters are queried or entered during the calls:

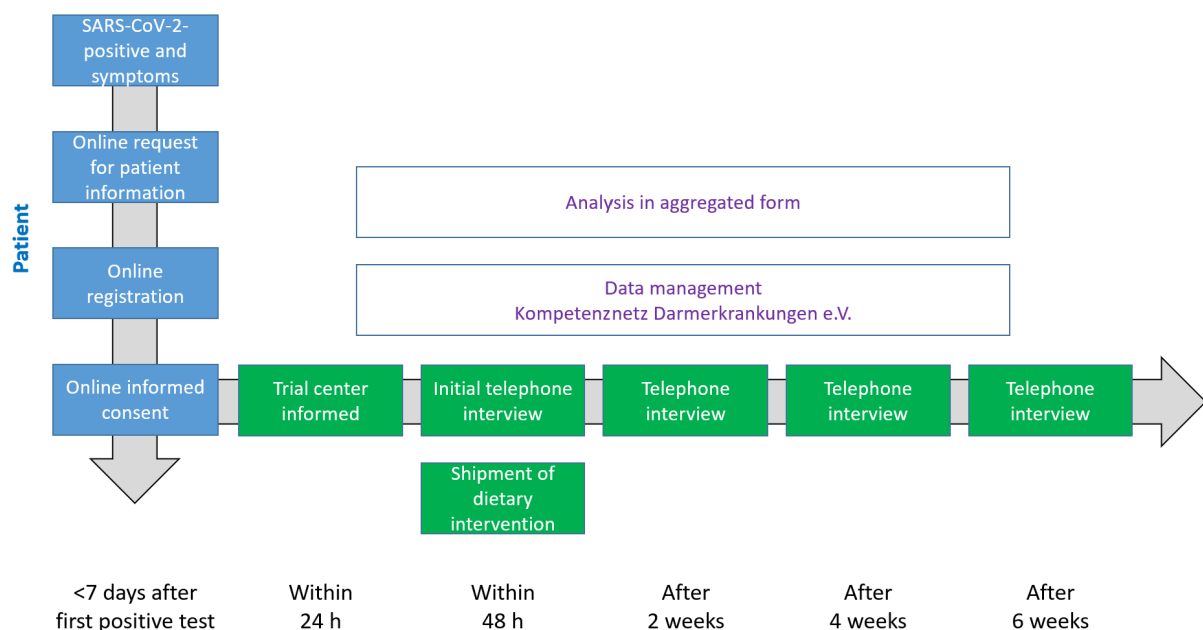
Week 0:

- Personal and demographic data
- COVID-19-relevant and other symptoms
- Inclusion criteria
- Comorbidities
- Smoking status
- Chronic medication and supplementation
- Hospitalization
- WHO scale for COVID-19
- Type of supplementation
- Epidemiological questions about living conditions

Weeks 2, 4 and 6:

- Intake of supplementation and other dietary supplements, if applicable.
- COVID-19-related and other symptoms
- Death and cause of death
- smoking status
- Examination in an emergency department
- Hospitalization
- WHO scale for COVID-19
- Ventilation requirement
- Intensive care treatment

The trial flow is summarized in the following flow chart:



8.3 Trial Duration

The trial duration for the individual patient is regularly 4 weeks until the end of the trial, 6 weeks if symptoms persist. A final interview takes place after approximately 6 months. This is particularly aimed at quality of life and possible late effects and also includes the EQ-5D-5L questionnaire (in the eCRF) as well as further paper questionnaires, an olfactory test and a cognition test (see section 5) analogous to the COVIDOM trial, in which the patients of the COVIt trial can also be included. In addition, patients may have a test performed for SARS-CoV-2 antibodies. The total duration of the trial will depend on the recruitment success and the further course of the COVID-19 epidemic. Enrollment has started on 01 February 2021. We anticipate enrolment of the last patient in late 2021 / early 2022.

9 Risk-Benefit Assessment

If the optimization of the nicotinamide status helps at least some of the COVID-19 patients to overcome the disease more quickly and/or more easily, then – in the repeatedly dramatically worsening situation – every patient who is not hospitalized or requires ventilation and every patient who recovers completely more quickly is a gain personally (intrinsic benefit) and also in relation to the totality of all COVID-19 patients (group benefit) and other patients in the hospital, as well as in relation to the socioeconomic consequences (sick days, late effects). The other extraneous benefit is a gain in knowledge for clinical research (improvement of patient care by a simple, immediately available and safe nutritional supplement). Risks are not recognizable.

Patient supplementation is discontinued when a critical condition is reached and the patient is admitted to a monitoring area.

10 Sample Size Calculation and Statistics

The available data at the start of the COVIt-2 trial suggested that at most 40% of the already symptomatic COVID-19 patients would be completely symptom-free two weeks after the start of the intervention (primary endpoint defined at that time). This would likely have been the case in the placebo-treated patient collective. The hypothesis was that this 40% event rate could be increased to 50% with the use of nicotinamide.

Statistical analysis is performed using SAS software version 9.3 or later (SAS Institute, Inc., Cary, North Carolina) or R. The statistical methodology is laid down in detail in a statistical analysis plan (SAP) in final form before the database lock. Any changes to the analysis plan in the protocol are documented in the SAP and presented in the final trial report. Continuous variables are summarized by descriptive statistics (number of observations (n), mean/median, standard deviation, minimum and maximum). Categorical variables are reported in frequency tables summarizing the number and percentage of patients in each category.

The intention-to-treat (ITT) population is defined as “all included patients”. The “all-randomized” (AR) population includes all patients who were observed for at least two weeks (intake of the dietary supplement and telephone interviews). The AR population is used to assess the primary and secondary endpoints. The biomarker (BM) population includes all patients from whom biomaterials could be obtained.

For sample size calculation, the standard variables were assumed to be: α -error = 0.05; power = 0.8; resulting in a $K = 7.85$ (factor calculated from α -error and power). The following simplified formula was used:

$$n = \frac{K [(R + 1) - p_2 (R^2 + 1)]}{p_2 (1 - R)^2}$$

n = required case number

p1 = Event rate in the treatment group (50%)

p2 = Event rate in the control group (40%)

R = relative risk (p1/p2) = here: 50% / 40% = 1.25

As a result, 385 patients per group were required. Based on previous experience, just under 10% were estimated as a safety margin for dropouts, therefore 420 patients per group should be included. If necessary, the case number calculation should be adjusted on the basis of the blinded event rate in the study.

The primary endpoint is analyzed using a generalized linear model with log-link and binomial error distribution. Results are presented as relative risk with 95% confidence interval. Binary secondary endpoints are analyzed using logistic regression models. Ordinal secondary endpoints (e.g. symptom severity) will be analyzed with proportional odds models with treatment group as the independent variable. In addition, stratifications will be made according to important prognostic factors such as symptom severity or specific symptoms at week 0 (baseline). The details are laid down in the SAP.

Based on the results of the futility analysis, the Data Management Board (DMB) recommended to the study management to maintain the previously planned enrollment size of approximately 840 patients and to select the subgroup of patients with at least one risk factor for severe COVID-19 progression as the primary clinical analysis population (see Section 11). To ensure that an adequate proportion of such patients is also included in the subpopulation recruited after futility analysis, the frequencies of risk factors will be monitored in a blinded manner and up to 10% more patients will be recruited as a safeguard against possible clustering of study dropouts.

11 Futility Analysis and Sample Size Adaptation

After 400 completed patients (with data collection including week 6), the frequency of the primary endpoint was assessed in the blinded data. When designing the COVit-2 study, it was expected that approximately 45% of patients should be symptom-free after 2 weeks of supplementation. If necessary, an interim evaluation and adjustment of the sample size with expansion or reduction of the number of patients should be performed.

For this planned futility analysis by the DMB of the COVit trial after collection of all data up to week 6 for 400 patients, a cleaned data set with n = 402 patients (201 each supplemented with nicotinamide or placebo, respectively) was available. In this data set, statistically significant positive effects of nicotinamide on certain subgroups were found by the external and independent statisticians of Novostat AG, which occurred most frequently and most strongly after 2 weeks.

For the patients recruited in the meantime, blinded and unadjusted frequencies of leading symptoms and affiliations to major risk groups were requested from the data management of the Competence Network Intestinal Diseases e.V. These indicated that the patient population from patient no. 403 onwards did not differ significantly from the one before. However,

because of the exclusion criterion of vaccination against SARS-CoV-2, increasing social selection is occurring.

The previous primary endpoint “complete resolution of symptoms after 2 weeks” and the corresponding secondary endpoints were based on the understanding of the disease course at the beginning of the COVit-2 trial and the, from today’s perspective, unusual distribution of risk factors in the patient cohort of the pilot trial COVit-1. It is now clear that the populations of COVit-1 and COVit-2 differ and that the complete symptom resolution achieved comparatively often in COVit-1 occurs much less frequently in patients in COVit-2. This is true both for the overall population and for subgroups with COVID-19 leading symptoms such as cough at week 0. Moreover, the frequency of these leading symptoms is lower in patients in COVit-2 than in COVit-1, which reduces the signal amplitude. On the other hand, the longer duration of illness observed in COVit-2 is consistent with current literature data, also relating to PCS. Based on the advice of the DMB, the PCS should be considered more strongly than before in the evaluation of the COVit-2 trial, especially since a meaningful battery of tests and questionnaires was added in the course of the trial.

In addition, the designs and initial results of clinical trials with orally administered drug candidates such as molnupiravir and PAXLOVID™ should be considered. Similar to nicotinamide, these compounds are intended to be used immediately after diagnosis of SARS-CoV-2 infection to mitigate disease progression. Unfortunately, such candidates did not exist at the time COVit-2 was planned. However, the studies of molnupiravir and PAXLOVID™ have included only patients with at least one risk factor for severe disease progression because these appear to benefit particularly from early intervention. The COVit-2 population as a whole, on the other hand, shows a disease that is too mild and too prolonged to perform meaningful evaluations with the previous strategy.

Recent findings on NAD metabolism in mature B cells (Bernardes et al. 2020, Immunity 53:1296) also suggest that the immunological response to SARS-CoV-2 should be investigated in addition to symptom-based analysis in the acute and post-acute disease course. It is quite conceivable that supplementation with nicotinamide could support antibody formation during infection by increasing the availability of NAD. Appropriate measurements of antibody levels at approximately 6 months are already part of the 6-month follow-up and should be upgraded.

The DMB therefore drew the following conclusions in its deliberations:

1. The continuation of the trial was and is warranted because patients have no discernible disadvantages from the supplementation and certain groups benefit significantly from nicotinamide supplementation.
2. The previously planned recruitment size of approx. 840 patients should be maintained. Since significantly higher effect sizes than previously assumed could be observed in individual subgroups with regard to COVID-19 symptoms or risk factors, highly significant signals could thus probably be expected in the total population of $n \geq 800$ (with a corresponding reserve of approx. 5% for study dropouts, hence 840). Even if divided into a hypothesis-generating population of the first $n = 402$ and a validation population of approximately equal size, some significant results would be expected according to the findings in the first $n = 402$.
3. As this is not a trial according to AMG, as the prerequisites for the trial have changed considerably in view of the recruited population, and as the current state of knowledge on suitable populations for COVID-19 studies in non-hospitalized patients should be

considered, the subgroup of patients with at least one risk factor for a severe course of COVID-19 is recommended as the primary clinical analysis population. The total population of $n \geq 800$ should serve only as a secondary analysis population.

4. It is recommended to convert the last two secondary endpoints (frequency and severity of symptoms) into the primary endpoint (frequency) and the first secondary endpoint (severity) and to maintain the endpoints regarding the time until complete resolution of symptoms as secondary endpoints. Furthermore, a focus on the frequency and severity after 2 weeks is recommended.
5. To adequately address the PCS, the DMB recommends upgrading the previously exploratory endpoint regarding the PCS into a secondary endpoint.
6. Based on current knowledge of B cell metabolism, the recommended immunological analysis population is the total population ($n \geq 800$), and the secondary immunological analysis population should be the subgroup of patients with at least one risk factor for a severe course of COVID-19. For possible effects of booster vaccination, stratification must be performed in both populations.
7. The levels of antibodies against SARS-CoV-2 should be investigated as a secondary endpoint.

12 Data Management, Data Protection and Handling of Biomaterials

Medical data incl. routine clinical findings will not be pseudonymized in this trial. However, a pseudonymization of the patient for the trial with a trial patient ID takes place as soon as the data are released for evaluation. In this process, the association of the patient data with the trial patient ID does not leave the recruitment center. The recruitment center is operated by the Competence Network Intestinal Diseases e.V., which also supports the trial in data management and by providing trained trial personnel for telephone data acquisition. Outside the recruitment center, only trial patient IDs will be handled.

A web-based Case Report Form (CRF) database set up for this trial will collect the information listed in Section 19.3 of the Appendix. In this process, data entry is blinded.

The data of the blood and stool analyses as well as the virus sequencing are stored pseudonymized in a digital database at the Institute for Clinical Molecular Biology (Kiel). For the blood tests at UKSH, study cases are created in ORBIS. The data storage will of course take place behind the firewall of CAU/UKSH and with all appropriate security precautions. Since the project is under medical management, all employees are subject to medical confidentiality. Any disclosure of data to unauthorized third parties (especially employers, insurance companies) is excluded. The transfer of samples and information to scientific cooperation partners takes place exclusively in pseudonymized form, i.e. without personal details. At the end of the research activities (after 20 years at the earliest), the samples and the associated data are destroyed. At the patient's request, they will receive a copy with a detailed description of the data management at the Institute of Clinical Molecular Biology. This complies with the analytical standards developed by the IHEC (International Human Epigenome Consortium; <http://ihc-epigenomes.org>). Before analysis of biosamples, these are recoded and linked to a pseudonymized data set.

Participation in this clinical trial is absolutely voluntary. Consent can be revoked by the patient at any time. This does not result in any disadvantages for the patients. Any samples already taken will then be destroyed immediately, and the data (except for data already

published/analyzed) will be deleted immediately, as far as technically possible (deletion after evaluation is no longer possible, the patient will also be informed of this). Patients may address any revocation of consent in writing to the project management of the Department of Internal Medicine I, University Medical Center Schleswig-Holstein, Campus Kiel, Rosalind-Franklin-Str. 12, Building K1, D-24105 Kiel.

It is possible that patents may arise in the course of this research project. In this case, there is no individual patent claim of the patients based on their individual data or individual biological materials.

13 Data Verification

Since the results of the telephone contacts are entered directly into the database, no systematic monitoring takes place. Exploratory data are only collected centrally and are subject to the data processing standards at the Institute for Clinical Molecular Biology (Kiel) described in section 12. If necessary, additional data will be requested from the attending physicians.

14 Protocol Changes after Trial Start

Protocol Amendments

Any changes to this protocol will be documented in a Protocol Amendment and require the approval of the Principal Investigator. These Protocol Amendments will be communicated to the relevant ethics committees and, in the case of substantial changes, will be submitted for prior approval.

Protocol Deviations

Deviations from this protocol should be avoided. If a protocol deviation occurs, the Principal Investigator will order a data reconciliation and the consequences of the deviation will be reviewed and discussed. Any deviation will be documented in the study file.

Early Termination of the Trial

The Principal Investigator has the right to terminate the trial at any time. In this case, the Principal Investigator must ensure that further decision-making is in the best interests of the patients. Termination of the trial will be reported to the appropriate ethics committee. The trial may be terminated by the responsible clinicians if

- the continuation of the study is not reasonable from an ethical or medical point of view,
- the resources to continue the study are not sufficiently available, or
- a meaningful outcome does not appear likely.

Individual trial participants whose health and safety are at risk may be excluded from the trial by participating clinicians at their discretion.

15 Ethical Aspects

Independent Ethics Committee

The Independent Ethics Committee of the Medical Faculty of CAU Kiel will evaluate this protocol as well as the subject information and subject consent, their updates (if any) and any other material given to the patients in writing.

End of Trial and End-of-Trial Notification

The end of trial is defined as the date on which the last trial participant is contacted by telephone at their last trial visit. At the end of the trial, the responsible ethics committee is notified.

Ethical Conduct of the Trial

This clinical trial will be conducted according to ethical principles following the Declaration of Helsinki and according to this approved protocol.

16 Subject Insurance

No subject or commuting accident insurance will be taken out for the trial. It is explicitly stated in the patient information that there is no subject commuting accident insurance. According to information provided by the UKSH insurance broker (Marsh Medical Consulting) and the responsible insurer (Berkshire Hathaway Specialty Insurance) on 03 December 2020, the UKSH public liability insurance covers the (extremely small) risks arising from the use of the non-marketed CICR-NAM within the scope of the trial.

17 Publication Rules

After completion of the trial, one or more manuscripts will be prepared for joint publications. Authorship will be assigned according to ICMJE criteria (for an official version, see <http://www.ICMJE.org>). The author list will follow the respective guidelines of the relevant journals or congresses. Contract laboratories do not have publication rights with respect to this trial.

18 Signature of the Principal Investigator

Kiel, _____

Prof. Dr. med. Stefan Schreiber

19 Appendix

19.1 Patient Information

19.2 Setup of the Online Informed Consent

19.3 Case Report Form (CRF)

19.4 Questionnaire Fatigue (FACIT-F)

19.5 Questionnaire Quality of Life (SF-36)

19.6 Additional Questionnaires for the Follow-Up after 6 Months

19.7 Additional Informed Consent for the Smell Test, Additional Questionnaires and Cognitive Test in the Follow-Up after 6 Months

19.8 Additional Informed Consent for Blood Sampling for Pharmacokinetics

19.9 Additional Informed Consent for the Antibody Test

KLINISCHES STUDIENPROTOKOLL

Verbesserung des Ernährungsstatus bezüglich Nicotinamid (Vitamin B3) und Verlauf der COVID-19-Erkrankung

Studienkürzel:
COVit-2

Version: 2.3

Datum: 13. Dezember 2021

Projektleiter: Prof. Dr. Stefan Schreiber

Klinik für Innere Medizin I

Universitätsklinikum Schleswig-Holstein, Campus Kiel

Arnold-Heller-Straße 3, Haus K1

24105 Kiel

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1 Zusammenfassung des Projekts

Aufgrund der Literatur erscheint es wahrscheinlich, dass eine Ernährungsintervention mit Nicotinamid (einer Form von Vitamin B3) die Therapie der SARS-CoV-2-Infektion (COVID-19) u.a. durch eine Verbesserung der Verfügbarkeit von Tryptophan und seiner stoffwechselunterstützenden Metaboliten (z.B. NAD) unterstützen kann. Eine Pilotphase der COVit-Studie (COVit-1) zeigte überraschend einen deutlichen Effekt von Nicotinamid auf die Zeit bis zum vollständigen Verschwinden der COVID-19-Symptome. Zudem ist Diarrhoe ein häufiges Symptom von COVID-19. Je 420 symptomatische Patienten mit bestätigter SARS-CoV-2-Infektion sollen daher in einem zweiten Teil der Studie für 4 Wochen verblindet pro Tag 1.000 mg Nicotinamid (500 mg herkömmliches Nicotinamid und 500 mg kontrolliert im Darm freigesetztes Nicotinamid) oder entsprechende Placebos einnehmen. Primärer Endpunkt der Studie ist das Auftreten einzelner COVID-19-Symptome zu Woche 0, Woche 2 (primärer Analysezeitpunkt), Woche 4 und Woche 6 sowie nach 6 Monaten. Sekundäre Endpunkte sind die Stärke von COVID-19-Symptomen, das Auftreten und die Stärke von Symptomen bei der 6-Monats-Nachverfolgung (Post-COVID-19-Syndrom, PCS), die Spiegel von Antikörpern gegen N-Protein und S-Protein von SARS-CoV-2 nach mindestens 6 Monaten, die vollständige Symptombefreiung nach 2, 4 und 6 Wochen sowie die Zeit von der Diagnose bis zur Freiheit von einzelnen oder allen Symptomen. Als explorative Endpunkte werden u.a. die klinische WHO-Skala für COVID-19 und eine Entwicklung von schwerem COVID-19 (Untersuchung in einer Notaufnahme, Hospitalisierung mit mindestens 24 Stunden Sauerstoffpflicht, Intensivpflicht, Beatmungspflichtigkeit oder Tod), Änderungen bei Fatigue und Lebensqualität sowie verschiedene Biomarker untersucht. Die Patienten werden nach positiver Testung angesprochen, aufgeklärt und können über eine Webseite ihre Teilnahme erklären. Nach randomisierter Austeilung der Studienmedikation werden die Patienten zu Beginn (Woche 0) und nach 2, 4 und 6 Wochen in Telefoninterviews zu ihrem Krankheitsverlauf befragt. Bei bis zu 400 Patienten werden zu Woche 0, Woche 2, Woche 4, Woche 6 und nach 6 Monaten Stuhlproben gewonnen. Zusätzlich werden bei bis zu 20 ausgewählten Patienten neben Blutbild und Standardblutprofil verschiedene Entzündungsmarker und das Metabolom, insbesondere der Tryptophanstoffwechsel, untersucht. Bei diesen Patienten wird auch der Virenstamm durch Sequenzierung aus Nasen-Rachen-Abstrichen bestimmt. Bei ausgewählten Patienten wird neben den genannten Blutwerten auch die Pharmakokinetik von Nicotinamid, Nicotinsäure und Nicotinursäure sowie von Metaboliten des Nicotinamid- und Tryptophanstoffwechsels in den ersten 48 Stunden nach einer einmaligen Gabe der Studienpräparate untersucht. Im Stuhl werden die Veränderungen von Mikrobiom (bei 100–300 Patienten) sowie Metagenom und Metabolom (bei einer Subgruppe) analysiert. Die Studie soll schnelle Ergebnisse dahingehend produzieren, ob eine Supplementation von Nicotinamid den Krankheitsverlauf von COVID-19 lindern kann. Darüber hinaus soll durch eine Nachbefragung, einen Riechtest, einen für Telefonbefragungen validierten Kognitionstest (T3MS) und einen Test auf SARS-CoV-2-Antikörper nach mindestens 6 Monaten untersucht werden, ob eine solche Supplementation einen Einfluss auf das PCS sowie die Immunreaktion und ggf. Impfung gegen SARS-CoV-2 hat.

2 Verantwortlichkeiten

Studienleiter:

Prof. Dr. med. Stefan Schreiber

Klinik für Innere Medizin I

Universitätsklinikum Schleswig-Holstein (UKSH), Campus Kiel

Arnold-Heller-Straße 3, Haus K1, 24105 Kiel

Tel.: 0431-500-15101, Email: s.schreiber@mucosa.de

Verantwortliche Zentren und Kontakte:

Studienzentren:

Klinik für Innere Medizin I, UKSH, Campus Kiel.

Zur Rekrutierung wird eine Vielzahl von Arztpraxen, Schwerpunktkrankenhäusern oder Teststellen/-laboren in Deutschland eingesetzt, die COVID-19-Patienten behandeln.

Beteiligte Studien-Labore:

Institut für Klinische Chemie, UKSH, Campus Kiel: Blutanalysen.

Fraunhofer ITEM (Hannover), MVZ Dr. Eberhard & Partner (Dortmund) und Klinikum der Universität München: Blutanalysen.

Institut für Klinische Molekularbiologie, CAU und UKSH, Campus Kiel: Mikrobiom-, Metagenom- und Metabolomanalysen im Stuhl; Blutanalysen; SARS-CoV-2-Sequenzierung. Adversis Pharma GmbH (Leipzig): Antikörperanalysen in Dried-Blood-Spot-Proben.

Telefonische Visiten:

Kompetenznetz Darmerkrankungen e.V., Kiel.

Datenhaltung und Statistik:

Kompetenznetz Darmerkrankungen e.V., Kiel: Datenhaltung und Statistik.

Novostat AG, Wollerau (CH): Externe Statistik (speziell Futility-Analyse).

Biobanking und Sample Management:

Institut für Klinische Molekularbiologie, CAU und UKSH, Campus Kiel.

Finanzierung:

Die Studie wird aus Eigenmitteln der Klinik für Innere Medizin I (UKSH, Campus Kiel), aus einer Anschubfinanzierung des Landes Schleswig-Holstein (Projekt DOI30) und vom Exzellenzcluster PMI finanziert. Das Studienprotokoll wurde vor Beginn der Rekrutierung im Deutschen Register Klinischer Studien (DRKS) registriert und wird nach zustimmender Bewertung entsprechend aktualisiert. Parallel dazu ist die Studie bei ClinicalTrials.gov registriert.

3 Wissenschaftlicher Hintergrund

Die Verfügbarkeit von Tryptophan und seiner Metabolite, insbesondere Nicotinamid (einer Form von Vitamin B3) als Baustein von NAD und NADP, sind wichtige Faktoren bei chronischen systemischen Entzündungen. Hier kann ein evolutionär alter Verteidigungsmechanismus fehlgeleitet werden, indem der Körper die Energienutzung in Zellen analog zur Eisenreduktion bei Entzündung drosselt (Boergeling & Ludwig 2017, FEBS J. 284:218). Während dies in einfachen Organismen Sinn ergibt, führen solche Mechanismen in komplexen Organismen zu Dysfunktionen (z.B. zu entzündungsbedingter Anämie). Reduzierte Spiegel an Nicotinamid und damit NAD(P) behindern die Immunabwehr gegen Coronaviren (Heer *et al.* 2020, J. Biol. Chem. 295:17986) und den Zellstoffwechsel, insbesondere auch der Makrophagen und Epithelzellen. Gerade letztere unterliegen einer kritisch hohen Erneuerungsrate im Darm und in der Lunge. Zudem kann Kynurenin als Hauptabbauprodukt des Tryptophans auch stark immunaktivierend wirken. Eine weitere Verbindung zwischen SARS-CoV-2 jenseits von Entzündung und Immunsystem könnte auch der SARS-CoV-2-Eintrittsrezeptor ACE2 sein, da die Anwesenheit von ACE2 auf der Zelloberfläche die Aufnahme von Tryptophan über den Rezeptor BOAT1 ermöglicht. Bei schweren Verläufen von Influenza werden ebenso wie bei chronischer Entzündung ein erhöhter Tryptophan-Abbau und damit erhöhte Kynurenin-Spiegel beobachtet, und Hemmung des Tryptophan-Abbaus hat im Tiermodell positive Effekte (Pizzini *et al.* 2019, Influenza Other Respir. Viruses 13:603; Boergeling & Ludwig 2017, FEBS J. 284:218). Im Gegenzug gibt es Hinweise bei unterschiedlichsten Virustypen, dass Nicotinamid die Virusreplikation reduzieren und die Abwehrmechanismen des Körpers unterstützen kann, z.B. bei Vaccinia (Child *et al.* 1988, Virus Res. 9:119), HIV (Murray 2003, Clin. Infect. Dis. 36:453), Enteroviren (Moell *et al.* 2009, J. Med. Virol. 81:1082) oder Hepatitis B (Li *et al.* 2016, Arch. Virol. 161:621). Auch für die Bekämpfung der Infektion mit SARS-CoV-2 wird eine hinreichende Versorgung mit B-Vitaminen und explizit Nicotinamid zur Stärkung des Immunsystems empfohlen (Zhang & Liu 2020, J. Med. Virol. 92:479; Gharote 2020, Ind. J. Med. Sci. 72:25; Shakoor *et al.* 2021, Maturitas 144:108). Hier wird besonders die Verbesserung der Abwehr bakterieller Sekundärinfektionen in Krankheitsmodellen hervorgehoben (Zhang & Liu 2020, J. Med. Virol. 92:479). Die in einer Studie beobachtete verminderte Sauerstoffsättigung bei einer Dosis von 400 mg/kg (dies entspräche einer wohl toxischen Dosis von 28 g bei einer 70-kg-Person) hat keine Aussagekraft für eine Nahrungsintervention mit 1.000 mg Gesamtdosis und angesichts des *acceptable daily intake* von 900 mg/Tag (EFSA Panel on Dietetic Products, Nutrition and Allergies 2014, EFSA J. 12:3759). In der COVit-Studie werden 1.000 mg verabreicht, was weit unter potentiell schädlichen Dosen von etlichen Gramm pro Tag und sehr nahe am ADI liegt [OECD-SIDS: 3-pyridinecarboxamide (nicotinamide), SIDS Initial Assessment Report for SIAM 15, Boston, Massachusetts, 22-25 October 2002]. Mittlerweile legen etliche Publikationen den Einsatz von Nicotinamid bei COVID-19 nahe, aber es gibt keine Studiendaten dazu (Shi *et al.* 2020, Cell Death Differ. 27:1451; Gharote 2020, Ind. J. Med. Sci. 72:25; Mehmehl *et al.* 2020, Nutrients 12:1616; Shakoor *et al.* 2021, Maturitas 144:108; Heer *et al.* 2020, J. Biol. Chem. 295:17986).

In der Pilotphase der COVit-Studie (COVit-1) erhielten zunächst je n=8 Patienten 1.000 mg herkömmliches Nicotinamid oder 245 mg Kieselerde als Placebo. Bei einer Qualitätskontrolle der Datenerhebung wurde überraschend beobachtet, dass 4 von 8 Patienten (50 %) aus der Nicotinamidgruppe bereits nach zweiwöchiger Gabe vollständig symptomfrei waren, während dies in der Kontrollgruppe nur bei einem der 8 Patienten (12,5 %) der Fall war. Die Auswertung der COVit-Vorstudie mit n=28 pro Gruppe zeigte eine signifikante Verkürzung der Zeit bis zur vollständigen Symptombefreiheit, die von Effekten bei Patientinnen getragen wurde. Aus der

Literatur waren je nach Patientenpopulation ebenfalls sehr lange Rekonvaleszenzen zu erwarten, z.B.

- 43 % Symptombefreiheit 14–21 Tage nach positivem SARS-CoV-2-Test (Tenforde et al. 2020, MMWR 69:99),
- <30 % ohne Atembeschwerden nach einem Monat (Marshall 2020, Nature 585:339),
- 32 % Symptombefreiheit nach 30 Tagen und immer noch nur 34 % nach 60 Tagen nach SARS-CoV-2-Diagnose (Carvalho-Schneider et al. 2021, Clin. Microbiol. Infect. 27:258), oder
- nur 12,6 % Symptombefreiheit nach im Mittel 60 Tagen (Carfi et al. 2020, JAMA 324:604).

Zudem hat sich COVID-19 seit Beginn der ursprünglich geplanten COVit-Studie als Systemerkrankung mit einer unerwartet hohen gastrointestinalen Symptombhäufigkeit erwiesen (Mitsuyama et al. 2020, J. Clin. Med. 9:3630). Vor kurzem wurde publiziert, dass auch das Darmmikrobiom von COVID-19 deutlich und z.T. negativ beeinflusst wird (Yeoh et al. 2021, Gut 70:698). Daher werden in der Hauptphase der Studie (COVit-2) nun zwei verschiedene Tabletten mit je 500 mg Nicotinamid als Prüfpräparate eingesetzt: die herkömmlichen Nicotinamidtabletten mit unmittelbarer Freisetzung aus der Pilotphase und die UKSH-Eigenentwicklung CICR-NAM (*controlled-ileocolonic-release nicotinamide*), deren Tabletten das Nicotinamid verzögert und kontinuierlich ab dem unteren Dünndarm freisetzen. Dadurch soll die gastrointestinale positive Wirkung von Nicotinamid, speziell auch auf das Mikrobiom und dessen Interaktion mit dem Darm, erhöht werden, da das herkömmliche Nicotinamid sehr schnell in den Kreislauf aufgenommen wird (Fangmann et al. 2018, Diabetes Care 41:398). Vorexperimente mit einem CICR-NAM-Prototyp haben gezeigt, dass die systemische Exposition durch die neue Dosierung geringer sein wird als in der Pilotphase der Studie (Fangmann et al. 2018, Diabetes Care 41:398), in der die Exposition ohnehin schon nahe am ADI lag (s.o.). Damit wird das ohnehin minimale Risiko für Nebenwirkungen noch weiter reduziert.

Der wissenschaftliche Hintergrund hat sich im Laufe der Studie weiterentwickelt und im Rahmen der geplanten Futility-Analyse und Fallzahlüberprüfung zu einer Anpassung von Endpunkten und Analysen geführt. Dies wird ausführlich in Abschnitt 11 erläutert.

4 Projektziele

4.1 Primärziel und Hypothese

Das Primärziel der Studie ist die Untersuchung der Hypothese, dass COVID-19-Patienten unter realen Bedingungen einzelne COVID-19-Symptome schneller verlieren, wenn sie 1.000 mg Nicotinamid (500 mg herkömmliches Nicotinamid und 500 mg CICR-NAM) supplementieren.

4.2 Sekundärziele

Neben dem genannten Primärziel sind die Sekundärziele der Studie die genauere Untersuchung folgender Parameter: Stärke einzelner Symptome, Auftreten und Stärke von Symptomen bei der 6-Monats-Nachverfolgung (PCS), Spiegel von Antikörpern gegen N-Protein und S-Protein von SARS-CoV-2 nach mindestens 6 Monaten, vollständige Symptombefreiheit nach 2, 4 und 6 Wochen sowie die Zeit von der Diagnose bis zur Befreiung von einzelnen oder allen Symptomen.

4.3 Explorative Ziele und Fragestellungen

Explorativ sollen die klinische WHO-Skala für COVID-19 und eine Entwicklung von schwerem COVID-19 (Untersuchung in einer Notaufnahme, Hospitalisierung mit mindestens 24 Stunden Sauerstoffpflicht, Intensivpflicht, Beatmungspflichtigkeit oder Tod) untersucht werden. Änderungen bei Fatigue und Lebensqualität werden mittels validierter Fragebögen (FACIT-F und SF-36) analysiert. Weitere explorative Untersuchungen an bis zu 20 ausgewählten Patienten sollen Aufschluss über Blutbild, Standardblutprofil, verschiedene Entzündungsmarker und das Metabolom im Blut, insbesondere den Tryptophanstoffwechsel, geben. Bei diesen Patienten wird auch der Virenstamm durch Sequenzierung aus Nasen-Rachen-Abstrichen bestimmt. Bei ausgewählten Patienten kann sich eine detaillierte Pharmakokinetik anschließen. Im Stuhl werden die Veränderungen von Mikrobiom (bei 100–300 Patienten) sowie Metagenom und Metabolom (bei einer Subgruppe) analysiert.

5 Endpunkte

Der Krankheitsverlauf der Nicotinamid-supplementierten Gruppe und der Placebogruppe wird anhand folgender Endpunkte verglichen:

Primärer Endpunkt:

Auftreten einzelner COVID-19-Symptome zu Woche 0, Woche 2 (primärer Analysezeitpunkt), Woche 4 und Woche 6 sowie nach 6 Monaten.

Sekundäre Endpunkte:

1. Stärke einzelner COVID-19-Symptome zu Woche 0, Woche 2 (primärer Analysezeitpunkt), Woche 4 und Woche 6 sowie nach 6 Monaten.
2. Im Rahmen der 6-Monats-Nachverfolgung: Auftreten und Stärke von Symptomen, die charakteristisch für das PCS sind.

Hierzu wird vom Patienten selbst ein dafür validierter Riechtest (Smell Identification Test™; Sonsonics / MediSense) durchgeführt. Zudem werden neben den Fragebögen FACIT-F und SF-36 (wie während des eigentlichen Studienzeitraums von Woche 0 bis Woche 6) noch weitere Fragebögen (siehe Anhang 19.6.) zum Riech- und Schmeckvermögen (inkl. Questionnaire of Olfactory Disorders, QOD), zur Atmung (Multidimensionales Dyspnoe-Profil, MDP), zur seelischen Verfassung (Patient Health Questionnaire Depression, PHQ-8; Generalized Anxiety Disorder 7, GAD-7; Perceived Stress Scale, PSS; Brief Resilience Scale, BRS), zur Schlafqualität (Pittsburgh Sleep Quality Index, PSQI) und zur Fatigue (Multidimensional Fatigue Inventory, MFI) ausgefüllt. Zusätzlich wird ein für Telefonbefragungen validierter Kognitionstest (T3MS) durchgeführt.

3. Spiegel von Antikörpern gegen N-Protein und S-Protein von SARS-CoV-2 nach mindestens 6 Monaten (Anti-S-Protein stratifiziert nach Vorhandensein und Art einer Boosterimpfung).
4. Vollständige Symptommfreiheit nach 2 Wochen.
5. Vollständige Symptommfreiheit nach 4 Wochen.
6. Vollständige Symptommfreiheit nach 6 Wochen.

7. Zeit von der Diagnose bis zur Freiheit von einzelnen Symptomen (in Tagen, bis zu 6 Wochen).
8. Zeit von der Diagnose bis zur vollständigen Symptommfreiheit (in Tagen, bis zu 6 Wochen).

Folgende Symptome werden in Telefoninterviews abgefragt und in ihrem Verlauf einzeln explorativ untersucht: Fatigue / Abgeschlagenheit / Erschöpfung / Kraftlosigkeit, Leistungsabfall / eingeschränkte körperliche Leistungsfähigkeit, Fieber (bis 39 °C bzw. darüber), Schüttelfrost, Atemnot / Kurzatmigkeit, pfeifende oder keuchende Atmung, Lungenentzündung, Husten (mit oder ohne Auswurf / Sputumproduktion), Schnupfen / laufende Nase / Nasenschleimproduktion / Rhinorrhoea, Hals- / Rachenschmerzen oder Halskratzen, Heiserkeit, Muskelschmerzen (Myalgie), Gelenkschmerzen (Arthralgie), Gliederschmerzen, Brustschmerzen, Kopfschmerzen, Bauchschmerzen, Durchfall / Diarrhoe, Übelkeit, Erbrechen, Appetitlosigkeit / geringere Nahrungsaufnahme, weitere Magen-Darm-Symptome (vom Patienten individuell zu nennen), eingeschränkter Geruchssinn, eingeschränkter Geschmackssinn, Bewusstseinsstörungen / Verwirrtheit, Schwindel, Bindehautentzündung (Konjunktivitis), Hautausschlag, Haarausfall und andere Symptome (vom Patienten individuell zu nennen).

In einer Subgruppe von Patienten werden die täglichen Änderungen des Schweregrads einzelner Symptome während der ersten 4 Wochen explorativ untersucht; Abstufungen der Symptome: 0 = gar nicht, 1 = leicht, 2 = mäßig, 3 = schwer, 4 = unerträglich.

Zudem wird eine Beschwerdeskala für Infektionen der unteren Atemwege mit den Aspekten Husten, Schleimbildung, Kurzatmigkeit, Schlaf, Fähigkeit zur normalen Aktivität und allgemeines Krankheitsgefühl mit folgenden Abstufungen abgefragt: 0 = normal, 1 = sehr geringes Problem, 2 = geringes Problem, 3 = mäßig schlecht, 4 = schlecht, 5 = sehr schlecht, 6 = maximal schlecht.

Explorative Endpunkte:

1. World Health Organization (WHO) COVID-19 Ordinal Scale for Clinical Improvement (siehe <https://www.who.int/publications/i/item/covid-19-therapeutic-trial-synopsis>) zu Woche 0 (Baseline), Woche 2, Woche 4 und Woche 6 sowie nach 6 Monaten.
2. Auftreten von schwerem COVID-19, definiert als Erreichung eines der folgenden Charakteristika:
 - a. Untersuchung in einer Notaufnahme;
 - b. Hospitalisierung mit einer durchgehenden Sauerstoffpflicht von mindestens 24 Stunden;
 - c. Intensivpflicht;
 - d. Beatmungspflicht;
 - e. Tod durch COVID-19.
3. Vergleich zwischen den Befunden aus der Pilotphase der Studie und dem zweiten Studienteil.
4. Änderungen bei der Fatigue (Fragebogen FACIT-F).
5. Änderungen bei der Lebensqualität (Fragebogen SF-36).

6. Änderung der Spiegel von Tryptophan im Blut (bei ausgewählten Patienten).
7. Änderung der Spiegel von Tryptophan-Metaboliten im Blut (bei ausgewählten Patienten).
8. Änderung von Entzündungsmarkern (C-reaktives Protein, Interleukin-6, Ferritin, Neopterin, D-Dimere) im Blut (bei ausgewählten Patienten).
9. Änderungen im Blutbild und Standardblutprofil (bei ausgewählten Patienten).
10. Änderungen in der Zusammensetzung des Blutmetaboloms (bei ausgewählten Patienten).
11. Stamm des SARS-CoV-2-Virus (bei ausgewählten Patienten).
12. Änderungen in der Zusammensetzung des Stuhlmikrobioms (bei ausgewählten Patienten).
13. Änderungen in der Zusammensetzung des Stuhlmetagenoms (bei ausgewählten Patienten).
14. Änderungen in der Zusammensetzung des Stuhlmetaboloms (bei ausgewählten Patienten).
15. Pharmakokinetik (bei ausgewählten Patienten).

6 Studiendesign

Es handelt sich hierbei um eine monozentrische, randomisierte Studie, bei der symptomatische COVID-19-Patienten eine Nahrungsergänzung mit 1.000 mg Nicotinamid pro Tag in Tablettenform (1 x 500 mg NicoPel, IFC Deutschland/Derma Enzinger, und 1 x 500 mg CICR-NAM, NextPharma/UKSH) oder passende Placebotabletten (Fagron bzw. NextPharma/UKSH) erhalten. In der Pilotphase erhielt die Nicotinamid-Gruppe 2 x 500 mg NicoPel und die Kontrollgruppe 245 mg Kieselerde in einer Kapsel (1 x 245 mg Kieselerde-Kapsel, Twardy/Saluspharma). Die nicht perfekte Paarung von Verum und Placebo-ähnlicher Kontrolle war durch die Marktverfügbarkeit der Prüfpräparate und die enorme Dringlichkeit der Studie zu rechtfertigen. Es wird davon ausgegangen, dass die Vergleichsgruppe „Heilmittel Kieselerde“ eine mit der Placebobehandlung vergleichbare Effektstärke hatte, da Kieselerde überwiegend aus inertem Siliziumdioxid besteht. Allerdings sind auch für Kieselerde positive Aussagen über den Ernährungsbefit als bewertbare Beurteilungen etabliert, daher wurde das Placebo für den zweiten Studienteil optimiert. Eine doppelte Verblindung war und ist soweit wie möglich gewährleistet. Für die Patienten geht aus der Verpackung und Einnahmeanweisung des zugesandten Nahrungsergänzungsmittels nicht hervor, welches Präparat sie bekommen haben. Das datenerhebende Personal an den Telefonen wird per SOP angewiesen, die Art des Prüfpräparats nicht zu thematisieren und ist hinsichtlich der Gruppenzugehörigkeit der angerufenen Studienteilnehmer verblindet. Alle Patienten erhalten den üblichen klinischen Behandlungsstandard, die Nahrungsergänzung ist rein additiv zum Standard of Care.

7 Studienpopulation

Die zu untersuchende Studienpopulation von insgesamt bis zu ca. 840 Studienteilnehmern betrifft Patienten mit bestätigter SARS-CoV-2-Infektion und Symptomen, z.B. im Bereich der Atemwege und/oder des Gastrointestinaltrakts.

Die Einschlusskriterien sind:

- Durch Laborbefund gesicherte SARS-CoV-2-Infektion; der positive Test darf nicht mehr als 7 Tage zurückliegen.
- Einschlägige Infektsymptome, z.B. im Bereich der Atemwege oder des Gastrointestinaltrakts.
- Der Patient/die Patientin konnte vor Durchführung jeglicher Studien-Prozedur schriftlich über eine Webseite einwilligen und kann die studienabhängigen Voraussetzungen und Anforderungen einhalten.
- Der Patient/die Patientin ist volljährig (vollendetes 18. Lebensjahr).

Die Ausschlusskriterien sind:

- Aktuelle Teilnahme an einer anderen Studie.
- Schwangerschaft oder Stillen.
- Eine Impfung gegen SARS-CoV-2.

Die Rekrutierung findet in Kiel und in weiteren Arztpraxen, Schwerpunktkrankenhäusern und Teststellen/-laboren für SARS-CoV-2 in Deutschland statt.

8 Studienablauf

8.1 Aufklärung und Einwilligung

Die Therapieentscheidungen bei der Behandlung von COVID-19 liegen außerhalb des Studienprotokolls, d. h. die Indikationsstellung ist unabhängig und wird ohne Einfluss durch das Forschungsprogramm gestellt. Erst nach Indikationsstellung wird über eine mögliche Rekrutierung entschieden.

Alle Patienten in den jeweiligen Zentren (ambulant und stationär), welche alle Ein- und Ausschlusskriterien erfüllen, werden um eine Studienteilnahme gebeten. Eine studienspezifische Prozedur außerhalb des klinischen Standards im Screening erfolgt nicht. Die Patienten erhalten eine schriftliche Patienteninformation mit Aufklärung. Für Rückfragen und ein ärztliches Aufklärungsgespräch steht bei Bedarf eine Hotline zur Verfügung. Der Patient kann im Anschluss nach ausreichender Bedenkzeit online einwilligen und seine Einwilligung durch Ausdruck der entsprechenden Seite für sich dokumentieren (Probandeneinwilligung). Dieses Vorgehen ermöglicht auch die Teilnahme von Patienten in häuslicher Quarantäne. Erst nach der Online-Einwilligung erfolgt der erste Telefonkontakt zur Datenerhebung für Woche 0 (Baseline), bei dem zu Beginn die Fähigkeit zur Einwilligung des Patienten durch das Studienpersonal am Telefon noch einmal nach einer SOP überprüft wird. Auch hier kann der Patient weitere Rückfragen stellen und die ärztliche Hotline kann hinzugezogen werden.

Für den Riechtest, die zusätzlichen Fragebögen, den Kognitionstest und den Test auf SARS-CoV-2-Antikörper bei der 6-Monats-Nachverfolgung sowie für die pharmakokinetischen Messungen werden ergänzende schriftliche Einwilligungen der Patienten eingeholt.

8.2 Maßnahmen (Intervention/Kontrolle) und Zielgrößen

Nach der Einwilligung und Erfüllung aller Voraussetzungen werden die Patienten nach dem Zufallsprinzip dem Nicotinamid-Arm oder dem Placebo-Arm zugeordnet. Darüber wird der Patient aufgeklärt.

Nach der Randomisierung im Verhältnis 1:1 sind Kontroll-/Interviewanrufe zu Woche 0 (Baseline) sowie Woche 2, Woche 4 und Woche 6 geplant. Nach ca. 6 Monaten ist ein weiterer

Kontrollanruf zur Abklärung von Langzeitsymptomen vorgesehen. Zusammen mit den Tabletten erhalten die Patienten ausgedruckte Fragebögen zur Charakterisierung von Fatigue (FACIT-F) und Lebensqualität (SF-36), die sie selbst ausfüllen und per frankiertem Rückumschlag zurückschicken sollen. In diesem Zusammenhang können perspektivisch auch Wearables analog zum Votum A 101/20 zum Einsatz kommen, dies würde dann noch genauer spezifiziert und vorgelegt. Bis zu 400 Patienten erhalten Probenbehälter für Stuhlproben, die per Rückumschlag ans IKMB gesandt werden. Das Ziel ist es, 100–300 Probenserien für Stuhlmikrobiomanalysen zu erhalten, von denen ein repräsentativer Teil zusätzlich auf Änderungen im Stuhlmetagenom und Stuhlmetabolom untersucht wird. Bei voraussichtlich bis zu 20 Patienten aus Kiel und Umgebung (je bis zu 10 Patienten mit Verum und Placebo) werden zusätzlich Blutproben zu den Interviewzeitpunkten sowie zu Woche 1 genommen, um die explorativen Blutanalysen durchzuführen (siehe Endpunkte). Bei diesen Patienten wird zu Beginn der Untersuchungen auch der Virenstamm durch Sequenzierung aus Nasen-Rachen-Abstrichen bestimmt. Bei bis zu 5 ausgewählten Patienten aus dieser Gruppe wird außerdem zu einem späteren Zeitpunkt die Pharmakokinetik von Nicotinamid, Nicotinsäure und Nicotinursäure sowie von Metaboliten des Nicotinamid- und Tryptophanstoffwechsels in den ersten 48 Stunden nach einer einmaligen Gabe der Studienpräparate untersucht.

Darüber hinaus soll durch eine Nachbefragung, zusätzliche Fragebögen, einen Riechtest, einen Kognitionstest und einen Test auf SARS-CoV-2-Antikörper nach 6 Monaten untersucht werden, ob die Supplementation mit Nicotinamid einen Einfluss auf das PCS sowie die Immunreaktion und ggf. Impfung gegen SARS-CoV-2 hat. Die hier gewonnenen Informationen entsprechen (bis auf den zusätzlichen Antikörpertest) den im Rahmen der COVIDOM-Studie erhobenen Daten und erlauben eine spätere, separat zu begutachtende Vernetzung der beiden Studien.

Die Telefoninterviews werden vom Kompetenznetz Darmerkrankungen e.V. geführt. Bei medizinischen Fragen ruft ein Arzt aus der Klinik für Innere Medizin I zurück. Bei den Anrufen werden folgende Parameter abgefragt bzw. eingetragen:

Woche 0:

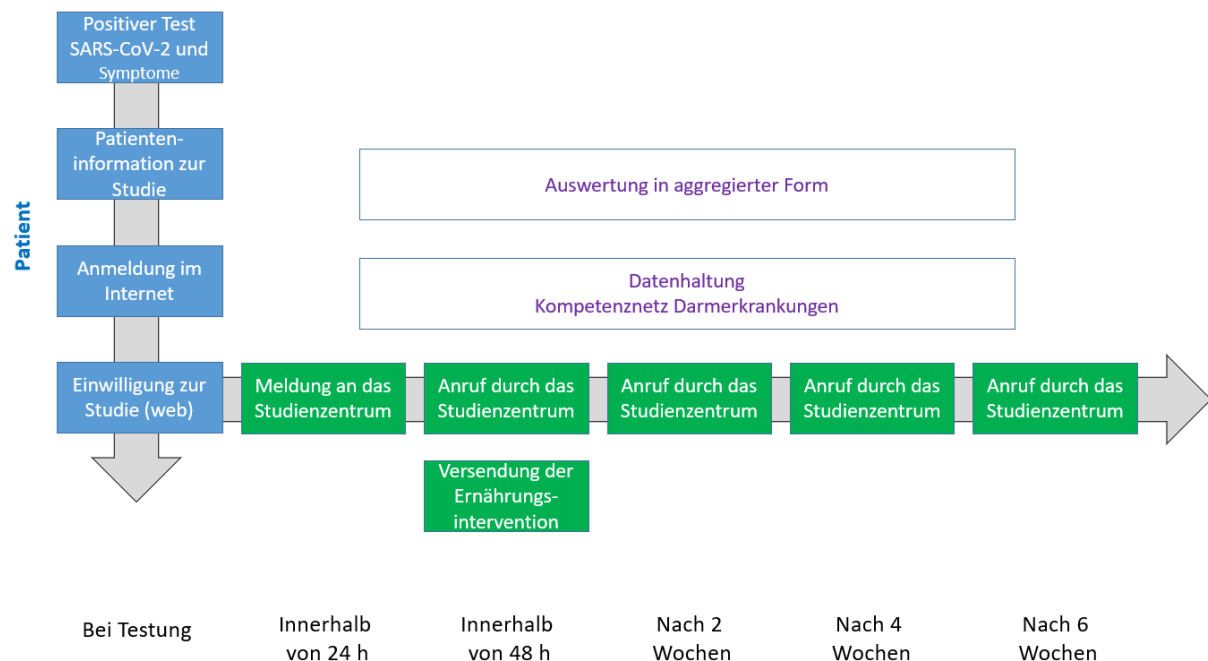
- Persönliche und demographische Daten
- COVID-19-relevante und andere Symptome
- Einschlusskriterien
- Komorbiditäten
- Raucherstatus
- Chronische Medikation und Supplementierung
- Hospitalisierung
- WHO-Skala zu COVID-19
- Art der Supplementierung
- Epidemiologische Fragen zu den Lebensumständen

Woche 2, 4 und 6:

- Einnahme der Supplementierung und ggf. anderer Nahrungsergänzungsmittel
- COVID-19-relevante und andere Symptome
- Tod und Todesursache
- Raucherstatus

- Untersuchung in einer Notaufnahme
- Hospitalisierung
- WHO-Skala zu COVID-19
- Beatmungspflicht
- Intensivstationäre Behandlung

Der Ablauf der Studie wird in folgendem Flow Chart zusammengefasst:



8.3 Studiendauer

Die Studiendauer für den einzelnen Patienten beträgt regulär 4 Wochen bis zum Studienende, bei persistierenden Symptomen 6 Wochen. Ein Abschlussgespräch erfolgt nach ca. 6 Monaten. Dieses zielt besonders auf die Lebensqualität und eventuelle Spätfolgen ab und umfasst auch den Fragebogen EQ-5D-5L (im eCRF) sowie weitere Papierfragebögen, einen Riechtest und einen Kognitionstest (siehe Abschnitt 5) analog zur COVIDOM-Studie, in welche die Patienten der COVit-Studie auch eingeschlossen werden können. Zudem können die Patienten einen Test auf SARS-CoV-2-Antikörper durchführen lassen. Die Gesamtdauer der Studie richtet sich nach dem Rekrutierungserfolg und dem weiteren Verlauf der COVID-19-Epidemie. Die Rekrutierung hat am 01.02.2021 begonnen. Wir rechnen mit einem Einschluss des letzten Patienten Ende 2021 / Anfang 2022.

9 Nutzen-Risiko-Abwägung

Wenn die Optimierung des Nicotinamid-Status zumindest einem Teil der COVID-19-Erkrankten dabei hilft, die Krankheit schneller und/oder leichter zu überwinden, ist bei der sich immer wieder dramatischen zuspitzenden Lage jeder nicht hospitalisierte oder beatmungspflichtige und jeder schneller vollständig gesundete Patient persönlich (Eigennutzen) und auch bezogen auf die Gesamtheit aller COVID-19-Patienten (Gruppennutzen) und anderen Patienten im Krankenhaus sowie bezogen auf die

sozioökonomischen Folgen (Krankheitstage, Spätfolgen) ein Gewinn. Der weitere Fremdnutzen besteht in einem Erkenntnisgewinn für die klinische Forschung (Verbesserung der Krankenversorgung durch ein einfaches, sofort verfügbares und sicheres Nahrungsergänzungsmittel). Risiken sind nicht erkennbar.

Die Supplementation von Patienten wird bei Erreichen eines kritischen Zustands und Aufnahme des Patienten in einen Überwachungsbereich abgebrochen.

10 Fallzahlberechnung und Statistik

Die Datenlage zu Beginn der COVIt-2-Studie ließ annehmen, dass höchstens 40 % der bereits symptomatischen COVID-19-Patienten zwei Wochen nach Beginn der Intervention vollständig symptomfrei sein würden (damals festgelegter primärer Endpunkt). Dies wäre im Kollektiv der mit Placebo behandelten Patienten voraussichtlich der Fall gewesen. Die Hypothese war, dass diese Ereignisrate von 40 % durch den Einsatz von Nicotinamid auf 50 % gesteigert werden kann.

Die statistische Analyse wird mit SAS-Software Version 9.3 oder neuer (SAS Institute, Inc., Cary, North Carolina) bzw. mit R durchgeführt. Die statistische Methodologie wird im Detail in einem statistischen Analyseplan (SAP) in finaler Form vor dem Database-Lock niedergelegt. Jegliche Änderung des Analyseplans im Protokoll wird im SAP dokumentiert und im finalen Studienbericht dargestellt. Kontinuierliche Variablen werden mit einer deskriptiven Statistik zusammengefasst (Zahl der Beobachtungen (n), Mittelwert/Median, Standardabweichung, Minimum und Maximum). Kategorische Variablen werden in Frequency Tables berichtet, welche die Zahl und den Prozentsatz von Patienten in den jeweiligen Kategorien zusammenfassen.

Die „Intention-to-treat“ (ITT)-Population ist definiert als „alle eingeschlossenen Patienten“. Die „All-Randomized“ (AR)-Population umfasst alle Patienten, die für mindestens zwei Wochen beobachtet wurden (Einnahme des Nahrungsergänzungsmittels und Telefoninterviews). Die AR-Population wird für die Beurteilung der primären und sekundären Endpunkte genutzt. Die Biomarker (BM)-Population umfasst alle Patienten, bei denen Biomaterialien asserviert werden konnten.

Für die Fallzahlplanung wurden als Standardgrößen angenommen: α -Fehler = 0,05; Power = 0,8; es ergibt sich ein $K = 7,85$ (Faktor, der sich aus α -Fehler und Power berechnet). Die folgende vereinfachte Formel wurde benutzt:

$$n = \frac{K [(R + 1) - p_2 (R^2 + 1)]}{p_2 (1 - R)^2}$$

n = benötigte Fallzahl

p1 = Ereignisrate in der Behandlungsgruppe (50 %)

p2 = Ereignisrate in der Kontrollgruppe (40 %)

R = relatives Risiko (p1/p2) = hier: 50 % / 40 % = 1,25

Im Ergebnis wurden 385 Patienten pro Gruppe benötigt. Als Sicherheitsreserve für Dropouts wurden aufgrund der bisherigen Erfahrungen knapp 10 % veranschlagt, daher sollten 420 Patienten pro Gruppe eingeschlossen werden. Die Fallzahlberechnung sollte ggf. anhand der verblindet ermittelten Ereignisrate in der Studie angepasst werden.

Der primäre Endpunkt wird unter Verwendung eines generalisierten linearen Modells mit Log-Link und binomialer Fehlerverteilung analysiert. Die Ergebnisse werden als relatives Risiko mit 95%-Konfidenzintervall dargestellt. Binäre sekundäre Endpunkte werden mit logistischen Regressionsmodellen analysiert. Ordinale sekundäre Endpunkte (z.B. Symptomschwere) werden mit Proportional-Odds-Modellen mit der Behandlungsgruppe als unabhängiger Variablen analysiert. Zusätzlich werden Stratifizierungen nach wichtigen prognostischen Faktoren wie Symptomschwere oder bestimmten Symptomen zu Woche 0 (Baseline) vorgenommen. Die Details sind im SAP niedergelegt.

Aufgrund der Ergebnisse der Futility-Analyse hat das Data Management Board (DMB) der Studienleitung empfohlen, den bislang geplanten Rekrutierungsumfang von ca. 840 Patienten beizubehalten und als primäre klinische Analysepopulation die Subgruppe von Patienten mit mindestens einem Risikofaktor für einen schweren COVID-19-Verlauf zu wählen (siehe Abschnitt 11). Um sicherzustellen, dass auch in der nach der Futility-Analyse rekrutierten Teilpopulation ein adäquater Anteil solcher Patienten enthalten ist, werden die Frequenzen der Risikofaktoren verblindet überwacht und zur Absicherung gegen eine eventuelle Häufung von Studienabbrüchen bis zu 10 % Patienten mehr rekrutiert.

11 Futility-Analyse und Anpassung der Stichprobengröße

Nach 400 abgeschlossenen Patienten (mit Datenerhebung bis inkl. Woche 6) wurde die Frequenz des primären Endpunkts in den verblindeten Daten beurteilt. Bei der Planung der COVit-2-Studie wurde erwartet, dass dann ca. 45 % der Patienten nach 2 Wochen Nahrungsergänzung symptomfrei sein sollten. Gegebenenfalls sollte eine Zwischenauswertung und eine Anpassung der Stichprobengröße mit Erweiterung oder Verringerung der Patientenzahl erfolgen.

Für diese geplante Futility-Analyse durch das DMB der COVit-Studie nach Erhebung aller Daten bis Woche 6 für 400 Patienten stand ein bereinigter Datensatz mit $n = 402$ Patienten (je 201 mit Nicotinamid bzw. Placebo supplementiert) zur Verfügung. In diesem Datensatz wurden von den externen und unabhängigen Statistikern der Firma Novostat AG statistisch signifikante positive Effekte von Nicotinamid auf bestimmte Subgruppen gefunden, die am häufigsten und stärksten nach 2 Wochen auftraten.

Für die zwischenzeitlich weiter rekrutierten Patienten wurden beim Datenmanagement des Kompetenznetzes Darmerkrankungen e.V. verblindete und unbereinigte Frequenzen von Leitsymptomen und Zugehörigkeiten zu größeren Risikogruppen angefordert. Diese wiesen darauf hin, dass sich das Patientengut ab Patient Nr. 403 nicht wesentlich von dem davor unterscheidet. Aufgrund des Ausschlusskriteriums einer Impfung gegen SARS-CoV-2 findet allerdings eine zunehmende soziale Selektion statt.

Der bisherige primäre Endpunkt „vollständige Symptommfreiheit nach 2 Wochen“ und die entsprechenden sekundären Endpunkte basierten auf dem Verständnis des Krankheitsverlaufs zu Beginn der COVit-2-Studie und der aus heutiger Sicht ungewöhnlichen Verteilung der Risikofaktoren in der Patientenkohorte der Vorstudie COVit-1. Mittlerweile ist klar, dass sich die Populationen von COVit-1 und COVit-2 unterscheiden und die in COVit-1 vergleichsweise oft erreichte vollständige Symptommfreiheit bei den Patienten in COVit-2 viel seltener auftritt. Dies gilt sowohl für die Gesamtpopulation als auch für Subgruppen mit COVID-19-Leitsymptomen wie z.B. Husten zu Woche 0. Zudem ist die Häufigkeit dieser Leitsymptome bei Patienten in COVit-2 geringer als in COVit-1, was die Signalamplitude verringert. Die in COVit-2 andererseits beobachtete längere Erkrankungsdauer deckt sich mit aktuellen Literaturdaten, auch zum PCS. Das PCS sollte auf Anraten des DMBs stärker als

bislang bei der Auswertung der COVit-2-Studie berücksichtigt werden, zumal hier im Studienverlauf noch eine aussagekräftige Batterie von Tests und Fragebögen hinzugekommen ist.

Ebenfalls neu zu berücksichtigen seien die Designs und ersten Ergebnisse klinischer Studien mit oral einnehmbaren Medikamentenkandidaten wie Molnupiravir und PAXLOVID™. Diese Substanzen sollen, ähnlich wie Nicotinamid, gleich nach der Diagnose einer SARS-CoV-2-Infektion eingesetzt werden, um Krankheitsverläufe abzumildern. Solche Kandidaten gab es zum Zeitpunkt der Planung von COVit-2 leider noch nicht. Die Studien zu Molnupiravir und PAXLOVID™ haben jedoch nur Patienten mit mindestens einem Risikofaktor für einen schweren Krankheitsverlauf einbezogen, weil diese besonders von einer frühzeitigen Intervention zu profitieren scheinen. Die Gesamtpopulation von COVit-2 ist hingegen zu leicht und zu lange erkrankt, um mit der bisherigen Strategie sinnvolle Auswertungen zu betreiben.

Neuere Erkenntnisse zum NAD-Metabolismus reifer B-Zellen (Bernardes et al. 2020, Immunity 53:1296) deuten zudem darauf hin, dass neben der symptombezogenen Analyse im akuten und postakuten Krankheitsverlauf auch die immunologische Antwort auf SARS-CoV-2 untersucht werden sollte. Es ist gut vorstellbar, dass eine Supplementation mit Nicotinamid durch die erhöhte Verfügbarkeit von NAD die Antikörperbildung während der Infektion unterstützen könnte. Entsprechende Messungen der Antikörperspiegel nach ca. 6 Monaten sind bereits Teil der 6-Monats-Nachverfolgung und sollten aufgewertet werden.

Das DMB hat bei seinen Beratungen daher folgende Schlussfolgerungen gezogen:

1. Die Weiterführung der Studie war und ist geboten, weil die Patienten keine erkennbaren Nachteile durch die Supplementation haben und bestimmte Gruppen von der Supplementation mit Nicotinamid signifikant profitieren.
2. Der bislang geplante Rekrutierungsumfang von ca. 840 Patienten sollte beibehalten werden. Da in einzelnen Subgruppen bezüglich COVID-19-Symptomen bzw. Risikofaktoren wesentlich höhere Effektstärken als bislang angenommen beobachtet werden konnten, wären damit voraussichtlich hochsignifikante Signale in der Gesamtpopulation von $n \geq 800$ (mit entsprechender Reserve von ca. 5 % für Studienabbrüche, daher 840) zu erwarten. Auch bei einer Aufteilung in eine hypothesengenerierende Population der ersten $n = 402$ und eine ca. gleich große Validierungspopulation wären gemäß den Befunden in den ersten $n = 402$ einige signifikante Ergebnisse zu erwarten.
3. Da es sich nicht um eine Studie nach AMG handelt, sich die Voraussetzungen für die Studie angesichts der rekrutierten Population stark geändert haben und da der aktuelle Wissensstand zu geeigneten Populationen für COVID-19-Studien bei nicht hospitalisierten Patienten berücksichtigt werden sollte, wird als primäre klinische Analysepopulation die Subgruppe von Patienten mit mindestens einem Risikofaktor für einen schweren COVID-19-Verlauf empfohlen. Die Gesamtpopulation von $n \geq 800$ sollte nur als sekundäre Analysepopulation dienen.
4. Es wird empfohlen, die bislang letzten beiden sekundären Endpunkte (Häufigkeit und Schwere von Symptomen) zum primären (Häufigkeit) und ersten sekundären Endpunkt (Schwere) zu machen und die Endpunkte zur Zeit bis zur vollständigen Symptommfreiheit als untergeordnete sekundäre Endpunkte weiterzuführen. Weiterhin ist eine Konzentration auf die Häufigkeit bzw. Schwere nach 2 Wochen empfehlenswert.

5. Um dem PCS angemessen Rechnung zu tragen, empfiehlt das DMB das Hochstufen des bislang explorativen Endpunkts zum PCS zu einem sekundären Endpunkt.
6. Aufgrund aktueller Erkenntnisse zum B-Zell-Metabolismus wird als immunologische Analysepopulation die Gesamtpopulation ($n \geq 800$) empfohlen, als nachgeordnete immunologische Analysepopulation sollte die Subgruppe von Patienten mit mindestens einem Risikofaktor für einen schweren COVID-19-Verlauf dienen. Für evtl. Effekte einer Boosterimpfung muss in beiden Populationen stratifiziert werden.
7. Die Höhe der Antikörperspiegel gegen SARS-CoV-2 sollte als sekundärer Endpunkt untersucht werden.

12 Datenmanagement, Datenschutz und Umgang mit Biomaterialien

Die medizinischen Daten inkl. klinischer Routine-Befunde werden in dieser Studie nicht pseudonymisiert. Es erfolgt jedoch eine Pseudonymisierung des Patienten für die Studie mit einer Studien-Patienten-ID, sobald die Daten zur Auswertung freigegeben werden. Die Zuordnung der Patientenklardaten mit der Studien-Patienten-ID verlässt dabei das Rekrutierungszentrum nicht. Das Rekrutierungszentrum wird vom Kompetenznetz Darmerkrankungen e.V. betrieben, das die Studie zudem bei der Datenhaltung und durch die Gestellung geschulten Studienpersonals für die telefonische Datenakquise unterstützt. Außerhalb des Rekrutierungszentrums wird ausschließlich mit Studien-Patienten-IDs umgegangen.

In einer für diese Studie eingerichteten, web-basierten Case Report Form (CRF)-Datenbank werden die in Abschnitt 19.3 des Anhangs aufgeführten Informationen gesammelt. Dabei erfolgt die Eingabe der Daten verblindet.

Die Daten der Blut- und Stuhl-Analysen sowie der Virus-Sequenzierungen werden im Institut für Klinische Molekularbiologie (Kiel) in einer digitalen Datenbank pseudonymisiert gespeichert. Für die Blutuntersuchungen am UKSH werden Studienfälle im ORBIS angelegt. Die Datenspeicherung erfolgt selbstverständlich hinter der Firewall von CAU/UKSH und mit allen entsprechenden Sicherheitsvorkehrungen. Da das Projekt unter ärztlicher Leitung steht, unterliegen alle Mitarbeiter der ärztlichen Schweigepflicht. Eine Weitergabe der Daten an unberechtigte Dritte (insbesondere Arbeitgeber, Versicherungen) ist ausgeschlossen. Die Weitergabe von Proben und Informationen an wissenschaftliche Kooperationspartner erfolgt ausschließlich in pseudonymisierter Form, d.h. ohne Angaben zur Person. Bei Beendigung der Forschungsaktivitäten (frühestens nach 20 Jahren) werden die Proben und die dazu gehörenden Daten vernichtet. Auf Patientenwunsch erhalten diese eine Kopie mit ausführlicher Darstellung des Datenmanagements am Institut für Klinische Molekularbiologie. Diese entspricht den analytischen Standards, welche vom IHEC (International Human Epigenome Consortium; <http://ihec-epigenomes.org>) entwickelt wurden. Vor Analyse von Bioproben werden diese rekodiert und mit einem pseudonymisierten Datensatz verbunden.

Die Teilnahme an dieser klinischen Studie ist absolut freiwillig. Die Zustimmung kann jederzeit vom Patienten widerrufen werden. Daraus entstehen den Patienten keinerlei Nachteile. Gegebenenfalls bereits entnommene Proben werden dann unverzüglich vernichtet, und die Daten (außer bereits veröffentlichte/analytierte Daten) werden umgehend gelöscht, soweit technisch möglich (eine Löschung nach Auswertung ist nicht mehr möglich, darüber wird der Patient auch informiert). Einen etwaigen Widerruf der Zustimmung können die Patienten schriftlich an die Projektleitung der Klinik für Innere Medizin I, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Rosalind-Franklin-Str. 12, Haus K1, D-24105 Kiel, richten.

Es kann sein, dass im Rahmen dieses Forschungsvorhabens Patente entstehen. In diesem Fall besteht kein individueller Patentanspruch der Patienten basierend auf deren individuellen Daten oder individuellen biologischen Materialien.

13 Datenverifizierung

Da die Eingabe der Ergebnisse der Telefonkontakte direkt in die Datenbank erfolgt, findet kein systematisches Monitoring statt. Explorative Daten werden nur zentral erhoben und unterliegen den in Abschnitt 12 beschriebenen Datenverarbeitungsstandards am Institut für Klinische Molekularbiologie (Kiel). Falls erforderlich, werden zusätzliche Daten von den behandelnden Ärzten abgefordert.

14 Protokolländerungen nach Studienbeginn

Protokoll-Amendments

Jede Veränderung dieses Protokolls wird in einem Protokoll-Amendment dokumentiert, und benötigt die Zustimmung des Studienleiters. Diese Protokoll-Amendments werden den zuständigen Ethikkommissionen mitgeteilt und im Falle wesentlicher Änderungen vorher zur Genehmigung vorgelegt.

Protokollabweichungen

Abweichungen von diesem Protokoll sollen vermieden werden. Falls eine Protokollabweichung stattfindet, beauftragt der Studienleiter einen Datenabgleich, und die Folgen der Abweichung werden überprüft und diskutiert. Jede Abweichung wird im Studienordner dokumentiert.

Frühzeitige Beendigung der Studie

Der Studienleiter hat das Recht, die Studie zu jeder Zeit zu beenden. In diesem Fall muss der Studienleiter sicherstellen, dass die weiteren Entscheidungen dem bestmöglichen Wohl der Patienten entsprechen. Die Beendigung der Studie wird der zuständigen Ethikkommission mitgeteilt. Die Studie kann durch die verantwortlichen Kliniker beendet werden, falls

- die Fortführung der Studie aus ethischer oder medizinischer Sicht nicht zumutbar ist,
- die Ressourcen zur Fortführung der Studie nicht suffizient vorhanden sind, oder
- ein aussagekräftiges Resultat nicht als wahrscheinlich erscheint.

Einzelne Studienteilnehmer, dessen Gesundheit und Sicherheit gefährdet ist, können von beteiligten Klinikern nach deren Ermessen von der Studie ausgeschlossen werden.

15 Ethische Aspekte

Unabhängige Ethik-Kommission

Die unabhängige Ethik-Kommission der medizinischen Fakultät der CAU Kiel wird dieses Protokoll sowie die Probandeninformation und Probandeneinwilligung, deren Aktualisierungen (falls vorhanden) und jedes weitere den Patienten schriftlich ausgehändigte Material evaluieren.

End-of-Trial und End-of-Trial-Benachrichtigung

Das End-of-Trial ist definiert als Datum, an dem der letzte Studienteilnehmer an seiner letzten Studienvisite telefonisch kontaktiert wird. Am Ende der Studie wird die zuständige Ethik-Kommission hierüber benachrichtigt.

Ethische Durchführung der Studie

Diese klinische Studie wird gemäß ethischen Prinzipien in Anlehnung an die Deklaration von Helsinki und nach diesem genehmigten Protokoll durchgeführt.

16 Probandenversicherung


Für die Studie wird keine Probanden- oder Wegeunfallversicherung abgeschlossen. In der Patienteninformation wird ausdrücklich darauf hingewiesen, dass keine Probanden- oder Wegeunfallversicherung besteht. Laut Auskunft des UKSH-Versicherungsmaklers (Marsh Medical Consulting) und des zuständigen Versicherers (Berkshire Hathaway Specialty Insurance) vom 03.12.2020 deckt die Betriebshaftpflichtversicherung des UKSH die (extrem geringen) Risiken aus der Verwendung des nicht auf dem Markt befindlichen CICR-NAM im Rahmen der Studie ab.

17 Publikationsregeln

Nach Beendigung der Studie werden ein oder mehrere Manuskripte für gemeinsame Publikationen erstellt. Die Autorenschaft wird nach ICMJE-Kriterien vergeben (für eine offizielle Version siehe <http://www.ICMJE.org>). Die Autorenliste richtet sich nach den jeweiligen Richtlinien der entsprechenden Fachzeitschriften oder Kongressen. Auftragslabore haben bezüglich dieser Studie kein Publikationsrecht.

18 Unterschrift des Studienleiters

Kiel, 13.12.2021


Prof. Dr. med. Stefan Schreiber

19 Anhang

19.1 Patienteninformation

19.2 Aufbau der Online-Einwilligung

19.3 Case Report Form (CRF)

19.4 Fragebogen Fatigue (FACIT-F)

19.5 Fragebogen Lebensqualität (SF-36)

19.6 Zusatzfragebögen zur Nachbefragung nach 6 Monaten

19.7 Ergänzende Einwilligung für den Riechtest, die Zusatzfragebögen und den Kognitionstest zur Nachbefragung nach 6 Monaten

19.8 Ergänzende Einwilligung für Blutabnahmen zur Pharmakokinetik

19.9 Ergänzende Einwilligung zum Antikörpertest

CLINICAL TRIAL PROTOCOL

Improvement of the Nutritional Status Regarding Nicotinamide (Vitamin B3) and the Disease Course of COVID-19

Trial Code:
COVit

Version: 1.1

Date: 26 March 2020

PI: Prof. Dr. Stefan Schreiber
Department of Internal Medicine I
University Medical Center Schleswig-Holstein, Campus Kiel
Arnold-Heller-Strasse 3, Building K1
24105 Kiel
Germany

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1 Summary of the Project

Based on the literature, it seems likely that a nutritional intervention with nicotinamide (a form of vitamin B3) can support the therapy of SARS-CoV-2 infection (COVID-19) by, *e.g.*, improving the availability of tryptophan and its metabolism-supporting metabolites (*e.g.* NAD). Five hundred patients each with confirmed SARS-CoV-2 infection and symptoms in the respiratory tract shall therefore self-administer 1,000 mg nicotinamide or 245 mg of the control substance silica per day in a blinded fashion for 4 weeks. The primary endpoint of the trial is the frequency of oxygen requirement, secondary endpoints are death, the frequencies of emergency department examination or hospitalisation and the time to resolution of symptoms. Patients are recruited after positive testing, receive the information for informed consent and can declare their participation via a website. After randomized distribution of the trial medication, the patients will be asked about their disease course in telephone interviews at baseline (week 0) and after 2, 4 and 6 weeks. From patients at UKSH giving global consent, serum samples to analyse levels of tryptophan, tryptophan metabolites and neopterin as well as stool samples to analyse the stool microbiome will be collected. The trial aims to generate rapid results on whether nicotinamide supplementation can alleviate the disease course of COVID-19.

2 Responsibilities

Principal Investigator of the trial:

Prof. Dr. med. Stefan Schreiber

Department of Internal Medicine I

University Medical Center Schleswig-Holstein (UKSH), Campus Kiel

Arnold-Heller-Strasse 3, Building K1, 24105 Kiel, Germany

Tel.: +49-431-500-15101, e-mail: s.schreiber@mucosa.de

Responsible centers and contacts:

Trial centers:

Department of Internal Medicine I, UKSH, Campus Kiel.

In addition, a large number of specialty hospitals or testing sites in Germany that treat COVID-19 patients will be contacted.

Participating trial laboratories:

Institute of Clinical Chemistry, UKSH, Campus Kiel (serum analyses).

Helmholtz GSF and Medical Center of the University of Munich (Germany) (serum analyses).

Institute of Clinical Molecular Biology, Kiel University and UKSH, Campus Kiel (stool microbiome analyses).

Telephone interviews:

Institute of Epidemiology (Popgen), Kiel University and UKSH, Campus Kiel (Prof. Dr. Wolfgang Lieb).

Competence Network Intestinal Diseases e.V., Kiel (Germany).

Data management and statistics:

Competence Network Intestinal Diseases e.V., Kiel (Germany).

Biobanking and sample management:

Institute of Clinical Molecular Biology, Kiel University and UKSH, Campus Kiel.

Funding:

The trial is funded by the Department of Internal Medicine I (UKSH, Campus Kiel). In parallel, attempts are being made to raise funds from the BMBF. The trial protocol will be registered in the German Register of Clinical Trials (DRKS) prior to the start of recruitment and will be registered at ClinicalTrials.gov.

3 Scientific Background

The availability of tryptophan and its metabolites, especially nicotinamide (a form of vitamin B3) as a building block of NAD and NADP, are important factors in chronic systemic inflammation. Here, an evolutionarily ancient defence mechanism can be misdirected by the body restricting energy use in cells analogous to iron reduction during inflammation (Boergeling & Ludwig 2017, FEBS J. 284:218). While this makes sense in simple organisms, such mechanisms lead to dysfunctions (e.g. inflammation-induced anaemia) in complex organisms. Reduced levels of nicotinamide and thus NAD(P) compromise cell metabolism, especially of macrophages and epithelial cells. The latter in particular are subject to a critically high rate of renewal in the gut and lungs. In addition, kynurenine, as the main degradation product of tryptophan, can also have a strong immune-activating effect. Another link between SARS-CoV-2 beyond inflammation and the immune system could also be the SARS-CoV-2 entry receptor ACE2, as the presence of ACE2 on the cell surface allows uptake of tryptophan via the receptor BOAT1. In severe courses of influenza, as in chronic inflammation, increased tryptophan degradation and thus increased kynurenine levels are observed, and inhibition of tryptophan degradation has beneficial effects in animal models (Pizzini et al. 2019, Influenza Other Respir. Viruses 13:603; Boergeling & Ludwig 2017, FEBS J. 284:218). In turn, there is evidence in a wide variety of virus types that nicotinamide can reduce viral replication and support the body's defence mechanisms, e.g. in vaccinia (Child et al. 1988, Virus Res. 9:119), HIV (Murray 2003, Clin. Infect. Dis. 36:453), enteroviruses (Moell et al. 2009, J. Med. Virol. 81:1082) or hepatitis B (Li et al. 2016, Arch. Virol. 161:621). Sufficient supply of B vitamins to strengthen the immune system is also recommended to combat SARS-CoV-2 infection (Zhang & Liu 2020, J. Med. Virol. 92:479). Here, the improvement of the defence against secondary bacterial infections in disease models is particularly emphasized (Zhang & Liu 2020, J. Med. Virol. 92:479). The reduced oxygen saturation observed in one study at a dose of 400 mg/kg (this would correspond to an arguably toxic dose of 28 g in a 70-kg person) has no significance for a dietary intervention with 1,000 mg total dose and given the acceptable daily intake of 900 mg/day (EFSA Panel on Dietetic Products, Nutrition and Allergies 2014, EFSA J. 12:3759). In the trial, 1,000 mg are administered, which is far below potentially harmful doses of several grams per day and very close to the ADI [OECD-SIDS: 3-pyridinecarboxamide (nicotinamide), SIDS Initial Assessment Report for SIAM 15, Boston, Massachusetts, 22-25 October 2002].

4 Project Objectives

4.1 Primary Objective and Hypothesis

The primary objective of the trial is to investigate the hypothesis that, under real clinical conditions, the frequency of severe disease courses in patients with COVID-19 decreases if they supplement 1,000 mg of nicotinamide.

4.2 Secondary Objectives and Exploratory Questions

In addition to the primary objective mentioned above, the secondary objectives of the trial are to investigate the frequencies of COVID-19-induced death, ventilator requirement, development of an ARDS and the time to complete resolution of symptoms in patients symptomatic at inclusion. Changes in serum levels of tryptophan, tryptophan metabolites and neopterin as well as in the stool microbiome between the beginning and the end of the intervention will be investigated in an exploratory fashion.

5 Endpoints

The disease course of the nicotinamide-supplemented group and the silica-supplemented group will be compared using the following endpoints:

Primary endpoint: frequency of hospitalization with a continuous oxygen requirement of at least 24 hours.

Secondary endpoints:

1. Frequency of ventilation requirement.
2. Frequency of death by COVID-19.
3. Frequency of examination in an emergency department.
4. Frequency of intensive care requirement.
5. Time from diagnosis to complete symptom resolution
6. Percentage of patients experiencing improvement of symptom severity at week 2 (endpoint 6A) or week 4 (endpoint 6B) (severe/moderate/mild).

Exploratory endpoints in UKSH patients:

1. Changes in serum levels of tryptophan.
2. Changes in serum levels of tryptophan metabolites.
3. Changes in serum levels of neopterin.
4. Changes in stool microbiome composition.

6 Trial Design

This is a monocentric, randomized trial in which symptomatic COVID-19 patients receive dietary supplementation with 1,000 mg nicotinamide per day in tablet form (2 x 500 mg NicoPel, IFC Germany/Derma Enzinger) or 245 mg silica in a capsule (1 x 245 mg silica capsule, Twardy/Saluspharma) in the control group. The less than perfect pairing of verum and placebo-like control is justified by the market availability of the investigational products and the tremendous urgency of the trial. Double blinding is ensured as far as possible. For the patients, it is not clear from the packaging and intake instructions of the dietary supplement sent to them which investigational product they received. Data collection personnel on the

telephones are instructed by SOP not to discuss the type of investigational product and are blinded as to the group allocation of the trial participants they are calling. All patients receive the usual clinical standard of care, the nutritional supplement is purely additive to the standard of care. It is assumed that the comparator group “remedy silica” has an effect size comparable to a placebo treatment, since silica consists mainly of inert silicon dioxide. However, positive statements on the nutritional benefit have also been established for silica as advertizable evaluations.

7 Trial Population

The trial population to be investigated, totalling 1,000 trial participants, involves patients with confirmed SARS-CoV-2 infection and symptoms in the respiratory tract.

Inclusion criteria are:

- SARS-CoV-2 infection confirmed by laboratory findings.
- Relevant infection symptoms in the respiratory or gastrointestinal tract (diarrhoea).
- The patient has been able to give written consent via a website before any trial procedure is performed and can comply with the trial-dependent prerequisites and requirements.
- The patient is of age (at least 18 years).

There are no exclusion criteria.

Recruitment will take place in Kiel, Germany, and in other specialty hospitals/testing sites for SARS-CoV-2 in Germany.

8 Trial Procedures

8.1 Informed Consent

Treatment decisions for COVID-19 are outside the trial protocol, i.e. the indication is independent and established without influence by the research program. Only after the indication has been established, a decision is made regarding possible recruitment.

All patients in the respective centers (outpatient and inpatient) who meet all inclusion and exclusion criteria will be asked to participate in the trial. There will be no trial-specific procedure outside the clinical standard in screening. Patients will receive a written patient information with informed consent. If necessary, a hotline is available for queries and a medical consultation for informed consent. After sufficient time for consideration, the patient can then consent online and document his or her consent by printing out the relevant page for him or herself (subject consent). This procedure also allows patients in quarantine at home to participate. Only after online consent, the first telephone contact is made for data collection for week 0 (baseline), wherein at the beginning the patient's ability to consent is checked again by study staff on the telephone according to an SOP. Again, the patient can ask further questions and the medical hotline can be consulted.

8.2 Measures (Intervention/Control) and Target Sizes

After consent and fulfilment of all requirements, patients are randomly assigned to the nicotinamide arm or the silica arm. The patient will be informed about this.

After randomization in a 1:1 ratio, control/interview calls are planned at week 0 (baseline) as well as week 2, week 4 and week 6. From patients at UKSH giving global consent, residual materials of serum and stool will be preserved for exploratory analyses of serum levels of

The telephone interviews are conducted by the Institute of Epidemiology (Popgen). For medical questions, a physician from the Department of Internal Medicine I calls back. The following parameters are queried or entered during the calls:

- Personal and demographic data
- COVID-19-relevant and other symptoms
- Inclusion criteria
- Comorbidities
- Smoking status
- Chronic medication and supplementation
- Hospitalization
- Type of supplementation
- Epidemiological questions about living conditions

- Intake of supplementation and other dietary supplements, if applicable.
- COVID-19-related and other symptoms
- Death and cause of death
- Smoking status
- Examination in an emergency department
- Hospitalization
- Ventilation requirement
- Intensive care treatment

The flowchart illustrates the study design, starting with a patient at testing. The process involves online registration, informed consent, and a trial center informed status. The patient then undergoes an initial telephone interview, followed by three more telephone interviews at 2, 4, and 6 weeks. The study also includes data management and analysis in aggregated form. A shipment of dietary intervention is provided within 48 hours of the initial interview.

```
graph TD
    Patient[Patient] --> SARS[SARS-CoV-2-positive and symptoms]
    SARS --> Request[Online request for patient information]
    Request --> Registration[Online registration]
    Registration --> Consent[Online informed consent]
    Consent --> Trial[Trial center informed]
    Trial --> Interview1[Initial telephone interview]
    Interview1 --> Interview2[Telephone interview]
    Interview2 --> Interview3[Telephone interview]
    Interview3 --> Interview4[Telephone interview]
    Interview4 --> End[ ]
    Interview1 --> Shipment[Shipment of dietary intervention]
    Consent --> Analysis[Analysis in aggregated form]
    Consent --> Data[Data management Kompetenznetz Darmkrankungen e.V.]
```

At testing

Within 24 h

Within 48 h

After 2 weeks

After 4 weeks

After 6 weeks

As part of the UKSH Global Consent for preserved residual biomaterials, serum and stool are preserved (only for UKSH patients).

8.3 Trial Duration

The trial duration for the individual patient is regularly 4 weeks until the end of the trial, potentially elongated to 6 weeks. A final interview takes place after approximately 6 months. The total duration of the trial will depend on the recruitment success and the further course of the COVID-19 epidemic. Enrolment shall start in April 2020. We anticipate enrolment of the last patient in summer 2020.

9 Risk-Benefit Assessment

If the optimization of the nicotinamide status helps at least some of the COVID-19 patients to overcome the disease more quickly and/or more easily, then – in the dramatically worsening situation – every patient who is not hospitalized or requires ventilation is a gain personally (intrinsic benefit) and also in relation to the totality of all COVID-19 patients (group benefit) and other patients in the hospital. The extraneous benefit is a gain in knowledge for clinical research (improvement of patient care by a simple, immediately available and safe nutritional supplement). Risks are not recognizable.

Patient supplementation is discontinued when a critical condition is reached and the patient is admitted to a monitoring area.

10 Sample Size Calculation and Statistics

The available data suggests that approximately 5% of already symptomatic COVID-19 patients could become hospitalized (due to oxygen therapy requirement) (primary endpoint). This would probably be the case in the silica-treated patient collective. The hypothesis is that this 5% event rate can be reduced to 2% with the use of nicotinamide.

Statistical analysis is performed using SAS software version 9.3 or later (SAS Institute, Inc., Cary, North Carolina). The statistical methodology is laid down in detail in a statistical analysis plan (SAP) in final form before the database lock. Any changes to the analysis plan in the protocol are documented in the SAP and presented in the final trial report. Continuous variables are summarized by descriptive statistics (number of observations (n), mean/median, standard deviation, minimum and maximum). Categorical variables are reported in frequency tables summarizing the number and percentage of patients in each category.

The intention-to-treat (ITT) population is defined as “all included patients”. The “all-randomized” (AR) population includes all patients who were observed for at least two weeks (intake of the dietary supplement and telephone interviews). The AR population is used to assess the primary and secondary endpoints. The biomarker (BM) population includes all patients from whom biomaterials could be obtained.

For sample size calculation, the standard variables were assumed to be: α -error = 0.05; power = 0.8; resulting in a $K = 7.85$ (factor calculated from α -error and power). The following simplified formula was used:

$$n = \frac{K [(R + 1) - p_2 (R^2 + 1)]}{p_2 (1 - R)^2}$$

n = required case number

p1 = Event rate in the treatment group (2%)

p2 = Event rate in the control group (5%)

R = relative risk (p1/p2) = here: 2% / 5% = 0.4

As a result, 607 patients per arm were required. The case number calculation may be adjusted on the basis of the blinded event rate in the study.

The primary endpoint is tested with a two-sided significance level of 5% in the ITT population using Fisher's Exact Test. Secondary endpoints are tested sequentially with the same test until significance at the 5% level is no longer achieved. Thereafter, these are exploratory and are only analysed descriptively.

11 Sample Size Adaptation

After 500 completed patients, the frequency of the primary endpoint will be assessed in the blinded data. It is expected that approximately 37 patients will then have received hospital care with mandatory oxygen. If necessary, the sample size will be adjusted by increasing the number of patients.

12 Data Management, Data Protection and Handling of Biomaterials

Medical data incl. routine clinical findings will not be pseudonymized in this trial. However, a pseudonymization of the patient for the trial with a trial patient ID takes place as soon as the data are released for evaluation. In this process, the association of the patient data with the trial patient ID does not leave the recruitment center. The recruitment center is operated and supervised by the Institute of Epidemiology, which is supported by the Competence Network Intestinal Diseases e.V. in terms of data management and by providing trained trial personnel for telephone data acquisition. Outside the recruitment center, only trial patient IDs will be handled.

A web-based Case Report Form (CRF) database set up for this trial will collect the information listed in Section 19.3 of the Appendix. In this process, data entry is blinded.

The data of the blood and stool analyses are stored pseudonymized in a digital database at the Institute for Clinical Molecular Biology (Kiel). The data storage will of course take place behind the firewall of CAU/UKSH and with all appropriate security precautions. Since the project is under medical management, all employees are subject to medical confidentiality. Any disclosure of data to unauthorized third parties (especially employers, insurance companies) is excluded. The transfer of samples and information to scientific cooperation partners takes place exclusively in pseudonymized form, i.e. without personal details. At the end of the research activities (after 20 years at the earliest), the samples and the associated data are destroyed. At the patient's request, they will receive a copy with a detailed description of the data management at the Institute of Clinical Molecular Biology. This complies with the analytical standards developed by the IHEC (International Human Epigenome Consortium; <http://ihec-epigenomes.org>). Before analysis of biosamples, these are recoded and linked to a pseudonymized data set.

Participation in this clinical trial is absolutely voluntary. Consent can be revoked by the patient at any time. This does not result in any disadvantages for the patients. Any samples already taken will then be destroyed immediately, and the data (except for data already published/analysed) will be deleted immediately, as far as technically possible (deletion after evaluation is no longer possible, the patient will also be informed of this). Patients may address any revocation of consent in writing to the project management of the Department of Internal Medicine I, University Medical Center Schleswig-Holstein, Campus Kiel, Rosalind-Franklin-Str. 12, Building K1, D-24105 Kiel.

It is possible that patents may arise in the course of this research project. In this case, there is no individual patent claim of the patients based on their individual data or individual biological materials.

13 Data Verification

Since the results of the telephone contacts are entered directly into the database, no systematic monitoring takes place. Exploratory data are only collected centrally and are subject to the data processing standards at the Institute for Clinical Molecular Biology (Kiel) described in section 12. If necessary, additional data will be requested from the attending physicians.

14 Protocol Changes after Trial Start

Protocol Amendments

Any changes to this protocol will be documented in a Protocol Amendment and require the approval of the Principal Investigator. These Protocol Amendments will be communicated to the relevant ethics committees and, in the case of substantial changes, will be submitted for prior approval.

Protocol Deviations

Deviations from this protocol should be avoided. If a protocol deviation occurs, the Principal Investigator will order a data reconciliation and the consequences of the deviation will be reviewed and discussed. Any deviation will be documented in the study file.

Early Termination of the Trial

The Principal Investigator has the right to terminate the trial at any time. In this case, the Principal Investigator must ensure that further decision-making is in the best interests of the patients. Termination of the trial will be reported to the appropriate ethics committee. The trial may be terminated by the responsible clinicians if

- the continuation of the study is not reasonable from an ethical or medical point of view,
- the resources to continue the study are not sufficiently available, or
- a meaningful outcome does not appear likely.

Individual trial participants whose health and safety are at risk may be excluded from the trial by participating clinicians at their discretion.

15 Ethical Aspects

Independent Ethics Committee

The Independent Ethics Committee of the Medical Faculty of CAU Kiel will evaluate this protocol as well as the subject information and subject consent, their updates (if any) and any other material given to the patients in writing.

End of Trial and End-of-Trial Notification

The end of trial is defined as the date on which the last trial participant is contacted by telephone at their last trial visit. At the end of the trial, the responsible ethics committee is notified.

Ethical Conduct of the Trial

This clinical trial will be conducted according to ethical principles following the Declaration of Helsinki and according to this approved protocol.

16 Subject Insurance

No subject or commuting accident insurance will be taken out for the trial. It is explicitly stated in the patient information that there is no subject or commuting accident insurance.

17 Publication Rules

After completion of the trial, one or more manuscripts will be prepared for joint publications. Authorship will be assigned according to ICMJE criteria (for an official version, see <http://www.ICMJE.org>). The author list will follow the respective guidelines of the relevant journals or congresses. Contract laboratories do not have publication rights with respect to this trial.

18 Signature of the Principal Investigator

Kiel, _____

Prof. Dr. med. Stefan Schreiber

19 Appendix

19.1 Patient Information

19.2 Setup of the Online Informed Consent

19.3 Case Report Form (CRF)

KLINISCHES STUDIENPROTOKOLL

Verbesserung des Ernährungsstatus bezüglich Nicotinamid (Vitamin B3) und Verlauf der COVID-19-Erkrankung

Studienkürzel:
COVit

Version: 1.1

Datum: 26. März.2020

Projektleiter: Prof. Dr. Stefan Schreiber

Klinik für Innere Medizin I

Universitätsklinikum Schleswig-Holstein, Campus Kiel

Arnold-Heller-Straße 3, Haus K1/K3

24105 Kiel

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 - 19.2. Aufbau der Online-Einwilligung
 - 19.3. Case Report Form (CRF)

1 Zusammenfassung des Projekts

Aufgrund der Literatur erscheint es wahrscheinlich, dass eine Ernährungsintervention mit Nicotinamid (einer Form von Vitamin B3) die Therapie der SARS-CoV-2-Infektion (COVID-19) u.a. durch eine Verbesserung der Verfügbarkeit von Tryptophan und seinen stoffwechselunterstützenden Metaboliten (z.B. NAD) unterstützen kann. Je 500 Patienten mit bestätigter COVID-19-Infektion und Symptomen im Bereich der Atemwege sollen daher für 4 Wochen verblindet pro Tag 1.000 mg Nicotinamid oder 245 mg der Kontrollsubstanz Kieselerde einnehmen. Primärer Endpunkt der Studie ist die Häufigkeit von Beatmungspflichtigkeit, sekundäre Endpunkte sind Tod, die Häufigkeiten einer Untersuchung in einer Notaufnahme oder Hospitalisierung und die Zeit bis zum Verschwinden der Symptome. Die Patienten werden nach positiver Testung angesprochen, aufgeklärt und können über eine Webseite ihre Teilnahme erklären. Nach randomisierter Austeilung der Studienmedikation werden die Patienten zu Beginn (Woche 0) und nach 2, 4 und 6 Wochen Telefoninterviews zu ihrem Krankheitsverlauf befragt. Bei Patienten am UKSH werden im Rahmen des Global Consent Serumproben zur Analyse der Spiegel von Tryptophan, Tryptophan-Metaboliten und Neopterin sowie Stuhlproben zur Analyse des Stuhlmikrobioms gesammelt. Die Studie soll schnelle Ergebnisse dahingehend produzieren, ob eine Supplementation von Nicotinamid den Krankheitsverlauf von COVID-19 lindern kann.

2 Verantwortlichkeiten

Studienleiter:

Prof. Dr. med. Stefan Schreiber

Klinik für Innere Medizin I

Universitätsklinikum Schleswig-Holstein (UKSH), Campus Kiel

Arnold-Heller-Straße 3, Haus K1, 24105 Kiel

Tel.: 0431-500-15101, Email: s.schreiber@mucosa.de

Verantwortliche Zentren und Kontakte:

Studienzentren:

Klinik für Innere Medizin I, UKSH, Campus Kiel.

Zudem wird eine Vielzahl von Schwerpunktkrankenhäusern oder Teststellen in Deutschland angeschrieben, die COVID-19-Patienten behandeln.

Beteiligte Studien-Labore:

Institut für Klinische Chemie, UKSH, Campus Kiel (Serumanalysen).

Helmholz GSF und Klinikum der Universität München (Serumanalysen).

Institut für Klinische Molekularbiologie, CAU und UKSH, Campus Kiel
(Stuhlmikrobiomanalysen).

Telefonische Visiten:

Institut für Epidemiologie (Popgen), CAU und UKSH, Campus Kiel (Prof. Dr. Wolfgang Lieb).
Kompetenznetz Darmerkrankungen e.V., Kiel.

Datenhaltung und Statistik:

Kompetenznetz Darmerkrankungen e.V., Kiel.

Biobanking und Sample Management:

Institut für Klinische Molekularbiologie, CAU und UKSH, Campus Kiel.

Finanzierung:

Die Studie wird aus Eigenmitteln der Klinik für Innere Medizin I (UKSH, Campus Kiel) finanziert. Parallel dazu wird versucht, Mittel vom BMBF einzuwerben. Das Studienprotokoll wird vor Beginn der Rekrutierung im Deutschen Register Klinischer Studien (DRKS) und unter ClinicalTrials.gov registriert.

3 Wissenschaftlicher Hintergrund

Die Verfügbarkeit von Tryptophan und seiner Metabolite, insbesondere Nicotinamid (einer Form von Vitamin B3) als Baustein von NAD und NADP, sind wichtige Faktoren bei chronischen systemischen Entzündungen. Hier kann ein evolutionär alter Verteidigungsmechanismus fehlgeleitet werden, indem der Körper die Energienutzung in Zellen analog zur Eisenreduktion bei Entzündung drosselt (Boergeling & Ludwig 2017, FEBS J. 284:218). Während dies in einfachen Organismen Sinn ergibt, führen solche Mechanismen in komplexen Organismen zu Dysfunktionen (z.B. entzündungsbedingte Anämie). Reduzierte Spiegel an Nicotinamid und damit NAD(P) behindern den Zellstoffwechsel, insbesondere auch der Makrophagen und Epithelzellen. Gerade letztere unterliegen einer kritisch hohen Erneuerungsrate im Darm und in der Lunge. Zudem kann Kynurenin als Hauptabbauprodukt des Tryptophan auch stark immunaktivierend wirken. Eine weitere Verbindung zwischen SARS-CoV-2 jenseits von Entzündung und Immunsystem könnte auch der SARS-CoV-2-Eintrittsrezeptor ACE2 sein, da die Anwesenheit von ACE2 auf der Zelloberfläche die Aufnahme von Tryptophan über den Rezeptor B0AT1 ermöglicht. Bei schweren Verläufen von Influenza werden ebenso wie bei chronischer Entzündung ein erhöhter Tryptophan-Abbau und damit erhöhte Kynurenin-Spiegel beobachtet, und Hemmung des Tryptophan-Abbaus hat im Tiermodell positive Effekte (Pizzini *et al.* 2019, Influenza Other Respir. Viruses 13:603; Boergeling & Ludwig 2017, FEBS J. 284:218). Im Gegenzug gibt es Hinweise bei unterschiedlichsten Virustypen, dass Nicotinamid die Virusreplikation reduzieren und die Abwehrmechanismen des Körpers unterstützen kann, z.B. bei Vaccinia (Child *et al.* 1988, Virus Res. 9:119), HIV (Murray 2003, Clin. Infect. Dis. 36:453), Enteroviren (Moell *et al.* 2009, J. Med. Virol. 81:1082) oder Hepatitis B (Li *et al.* 2016, Arch. Virol. 161:621). Auch für die Bekämpfung der Infektion mit SARS-CoV-2 wird ein hinreichender B-Vitamin-Status zur Stärkung des Immunsystems empfohlen (Zhang & Liu 2020, J. Med. Virol. 92:479). Hier wird besonders die Verbesserung der Abwehr bakterieller Sekundärinfektionen in Krankheitsmodellen hervorgehoben (Zhang & Liu 2020, J. Med. Virol. 92:479). Die in einer Studie beobachtete verminderte Sauerstoffsättigung bei einer Dosis von 400 mg/kg (dies entspräche einer wohl toxischen Dosis von 28 g bei einer 70-kg-Person) hat keine Aussagekraft für eine Nahrungsintervention mit 1.000 mg Gesamtdosis und angesichts des *acceptable daily intake* von 900 mg/Tag (EFSA Panel on Dietetic Products, Nutrition and Allergies 2014, EFSA J. 12:3759). In der Studie werden 1.000 mg verabreicht, was weit unter potentiell schädlichen Dosen von etlichen Gramm pro Tag und sehr nahe am ADI liegt [OECD-SIDS: 3-pyridinecarboxamide (nicotinamide), SIDS Initial Assessment Report for SIAM 15, Boston, Massachusetts, 22-25 October 2002].

4 Projektziele

4.1 Primärziel und Hypothesen

Das Primärziel der Studie ist die Untersuchung der Hypothese, dass unter realen klinischen Bedingungen die Häufigkeit der schweren Verläufe bei COVID-19-Erkrankten sinkt, wenn sie 1.000 mg Nicotinamid supplementieren.

4.2 Sekundärziele und explorative Fragestellungen

Neben dem genannten Primärziel sind die Sekundärziele der Studie die Untersuchung der Häufigkeit von COVID-19-induziertem Tod, die Häufigkeit einer Beatmung, die Häufigkeit der Entwicklung eines ARDS und die Zeit bis zur vollständigen Symptombefreiung der bei Einschluss symptomatischen Patienten. Explorativ sollen die Entwicklung der Spiegel von Tryptophan, Tryptophanmetaboliten und Neopterin im Serum sowie des Stuhlmikrobioms zwischen Beginn und Ende der Behandlung untersucht werden.

5 Endpunkte

Der Krankheitsverlauf der Nicotinamid-supplementierten Gruppe und der Kieselerde-supplementierten Kontrollgruppe wird anhand folgender Endpunkte verglichen:

Primärer Endpunkt: Häufigkeit einer Hospitalisierung mit einer durchgehenden Sauerstoffpflicht von mindestens 24 Stunden.

Sekundäre Endpunkte:

1. Häufigkeit von Beatmungspflicht.
2. Häufigkeit von Tod durch COVID-19.
3. Häufigkeit von Untersuchung in einer Notaufnahme.
4. Häufigkeit von Intensivpflicht.
5. Zeit von der Diagnose bis zur Symptombefreiung.
6. Prozentsatz der Patienten, die Verbesserung der Symptomschwere zur Woche 2 (Endpunkt 6A) oder Woche 4 (6B) erfahren (schwer/moderat/mild).

Explorative Endpunkte bei UKSH-Patienten:

1. Änderung der Spiegel von Tryptophan im Serum.
2. Änderung der Spiegel von Tryptophan-Metaboliten im Serum.
3. Änderung der Spiegel von Neopterin im Serum.
4. Änderung der Zusammensetzung des Stuhlmikrobioms.

6 Studiendesign

Es handelt sich hierbei um eine monozentrische, randomisierte Studie, bei der symptomatische COVID-19-Patienten eine Nahrungsergänzung mit 1.000 mg Nicotinamid pro Tag in Tablettenform (2 x 500 mg NicoPel, IFC Deutschland/Derma Enzinger) oder in der Kontrollgruppe 245 mg Kieselerde in einer Kapsel (1 x 245 mg Kieselerde-Kapsel, Twardy/Saluspharma) erhalten. Die nicht perfekte Paarung von Verum und Placebo-ähnlicher Kontrolle ist durch die Marktverfügbarkeit der Prüfpräparate und die enorme Dringlichkeit der Studie zu rechtfertigen. Eine doppelte Verblindung ist soweit wie möglich gewährleistet. Für die Patienten geht aus der Verpackung und Einnahmeanweisung des zugesandten

Nahrungsergänzungsmittels nicht hervor, welches Präparat sie bekommen haben. Das datenerhebende Personal an den Telefonen wird der SOP angewiesen, die Art des Prüfpräparats nicht zu thematisieren und ist hinsichtlich der Gruppenzugehörigkeit der angerufenen Studienteilnehmer verblindet. Alle Patienten erhalten den üblichen klinischen Behandlungsstandard, die Nahrungsergänzung ist rein additiv zum Standard of Care. Es wird davon ausgegangen, dass die Vergleichsgruppe „Heilmittel Kieselerde“ eine einer Placebobehandlung vergleichbare Effektstärke hat, da Kieselerde überwiegend aus inertem Siliziumdioxid besteht. Allerdings sind auch für Kieselerde positive Aussagen über den Ernährungsbefit als bewerbare Beurteilungen etabliert.

7 Studienpopulation

Die zu untersuchende Studienpopulation von insgesamt 1.000 Studienteilnehmern betrifft Patienten mit bestätigter SARS-CoV-2-Infektion und Symptomen im Bereich der Atemwege.

Die Einschlusskriterien sind hierbei:

- Durch Laborbefund gesicherte SARS-CoV-2-Infektion.
- Einschlägige Infektsymptome im Bereich der Atemwege oder des Gastrointestinaltrakts (Durchfall).
- Der Patient/die Patientin konnte vor Durchführung jeglicher Studien-Prozedur schriftlich über eine Webseite einwilligen und kann die Studien-abhängigen Voraussetzungen und Anforderungen einhalten.
- Der Patient/die Patientin ist volljährig (vollendetes 18. Lebensjahr).

Ausschlusskriterien gibt es nicht.

Die Rekrutierung findet in Kiel und in weiteren Schwerpunktkrankenhäusern/Teststellen für SARS-CoV-2 in Deutschland statt.

8 Studienablauf

8.1 Aufklärung und Einwilligung

Die Therapieentscheidungen bei der Behandlung von COVID-19 liegen außerhalb des Studienprotokolls, d. h. die Indikationsstellung ist unabhängig und wird ohne Einfluss durch das Forschungsprogramm gestellt. Erst nach Indikationsstellung wird über eine mögliche Rekrutierung entschieden.

Alle Patienten in den jeweiligen Zentren (ambulant und stationär), welche alle Ein- und Ausschlusskriterien erfüllen, werden um eine Studienteilnahme gebeten. Eine studienspezifische Prozedur außerhalb des klinischen Standards im Screening erfolgt nicht. Die Patienten erhalten eine schriftliche Patienteninformation mit Aufklärung. Für Rückfragen und ein ärztliches Aufklärungsgespräch steht bei Bedarf eine Hotline zur Verfügung. Der Patient kann im Anschluss nach ausreichender Bedenkzeit online einwilligen und seine Einwilligung durch Ausdruck der entsprechenden Seite für sich dokumentieren (Probandeneinwilligung). Dieses Vorgehen ermöglicht auch die Teilnahme von Patienten in häuslicher Quarantäne. Erst nach der Online-Einwilligung erfolgt der erste Telefonkontakt zur Datenerhebung für Woche 0, bei dem zu Beginn die Fähigkeit zur Einwilligung des Patienten durch das Studienpersonal am Telefon noch einmal nach einer SOP überprüft wird. Auch hier kann der Patient weitere Rückfragen stellen und die ärztliche Hotline kann hinzugezogen werden.

8.2 Maßnahmen (Intervention/Kontrolle) und Zielgrößen

Nach der Einwilligung und Erfüllung aller Voraussetzungen werden die Patienten nach dem Zufallsprinzip dem Nicotinamid-Arm oder dem Kieselerde-Arm zugeordnet. Darüber wird der Patient aufgeklärt.

Nach der Randomisierung im Verhältnis 1:1 sind Kontrollanrufe zu Woche 0, Woche 2, Woche 4 und Woche 6 geplant. Bei UKSH-Patienten werden im Rahmen des Global Consent Restmaterialien Serum und Stuhl für explorative Analysen der Serumspiegel von Tryptophan, Tryptophan-Metaboliten und Neopterin sowie der Zusammensetzung des Stuhlmikrobioms asserviert.

Die Telefoninterviews werden vom Institut für Epidemiologie (Popgen) geführt. Bei medizinischen Fragen ruft ein Arzt aus der Klinik für Innere Medizin I zurück. Bei den Anrufen werden folgende Parameter abgefragt bzw. eingetragen:

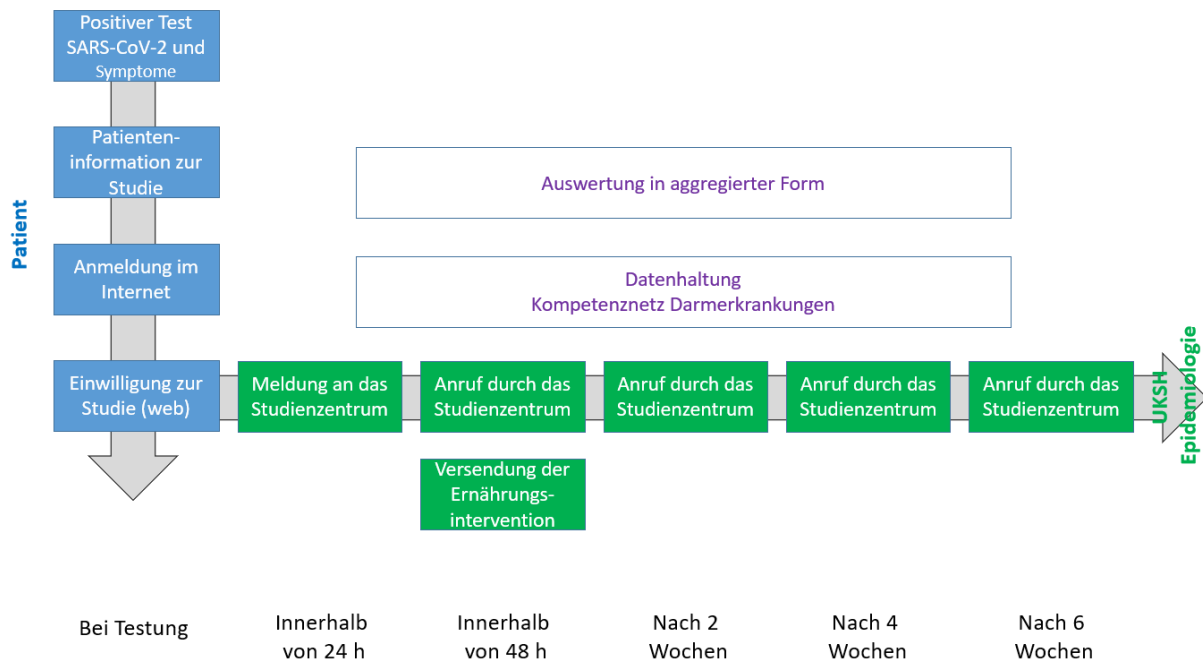
Woche 0:

- Persönliche und demographische Daten
- COVID-19-relevante und andere Symptome
- Einschlusskriterien
- Komorbiditäten
- Raucherstatus
- Chronische Medikation und Supplementierung
- Hospitalisierung
- Art der Supplementierung
- Epidemiologische Fragen zu den Lebensumständen

Woche 2, 4 und 6:

- Einnahme der Supplementierung und ggf. anderer Nahrungsergänzungsmittel
- COVID-19-relevante und andere Symptome
- Tod und Todesursache
- Raucherstatus
- Untersuchung in einer Notaufnahme
- Hospitalisierung
- Beatmungspflicht
- Intensivstationäre Behandlung

Der Ablauf der Studie wird in folgendem Flow Chart zusammengefasst:



Im Rahmen des UKSH-Global-Consent zu asservierten Rest-Biomaterialien werden Serum und Stuhl asserviert (nur bei UKSH-Patienten).

8.3 Studiendauer

Die Studiendauer für den einzelnen Patienten beträgt regulär 4 Wochen bis zum Studienende, ggf. mit Verlängerung auch 6 Wochen. Ein Abschlussgespräch erfolgt nach ca. 6 Monaten. Die Gesamtdauer der Studie richtet sich nach dem Rekrutierungserfolg und dem weiteren Verlauf der COVID-19-Epidemie. Die Rekrutierung soll im April 2020 starten. Wir rechnen mit einem Einschluss des letzten Patienten im Sommer 2020.

9 Nutzen-Risiko-Abwägung

Wenn die Optimierung des Nicotinamid-Status zumindest einem Teil der COVID-19-Erkrankten dabei hilft, die Krankheit schneller und/oder leichter zu überwinden, ist bei der sich dramatischen zuspitzenden Lage jeder nicht hospitalisierte oder beatmungspflichtige Patient persönlich (Eigennutzen) und auch bezogen auf die Gesamtheit aller COVID-19-Patienten (Gruppennutzen) und anderen Patienten im Krankenhaus ein Gewinn. Der Fremdnutzen besteht in einem Erkenntnisgewinn für die klinische Forschung (Verbesserung der Krankenversorgung durch ein einfaches, sofort verfügbares und sicheres Nahrungsergänzungsmittel). Risiken sind nicht erkennbar.

Die Supplementation von Patienten wird bei Erreichen eines kritischen Zustands und Aufnahme des Patienten in einen Überwachungsbereich abgebrochen.

10 Fallzahlberechnung und Statistik

Die bisherige Datenlage lässt annehmen, dass rund 5 % der bereits symptomatischen COVID-Patienten krankenhauspflchtig (wegen Bedarfs an Sauerstofftherapie) werden könnten (primärer Endpunkt). Dies wäre im Kollektiv der mit Kieselerde behandelten Patienten voraussichtlich der Fall. Die Hypothese ist, dass diese Ereignisrate von 5 % durch den Einsatz von Nicotinamid auf 2 % gesenkt werden kann.

Die statistische Analyse wird mit SAS-Software Version 9.3 oder neuer (SAS Institute, Inc., Cary, North Carolina) durchgeführt. Die statistische Methodologie wird im Detail in einem statistischen Analyseplan (SAP) in finaler Form vor dem Database-Lock niedergelegt. Jegliche Änderung des Analyseplans im Protokoll wird im SAP dokumentiert und im finalen Studienbericht dargestellt. Kontinuierliche Variablen werden mit einer deskriptiven Statistik zusammengefasst (Zahl der Beobachtungen (n), Mittelwert/Median, Standardabweichung, Minimum und Maximum). Kategorische Variablen werden in Frequency Tables berichtet, welche die Zahl und den Prozentsatz von Patienten in den jeweiligen Kategorien zusammenfassen.

Die „Intention-to-treat“ (ITT)-Population ist definiert als „alle eingeschlossenen Patienten“. Die „All-Randomized“ (AR)-Population umfasst alle Patienten, die für mindestens zwei Wochen beobachtet wurden (Einnahme des Nahrungsergänzungsmittels und Telefoninterviews). Die AR-Population wird für die Beurteilung der primären und sekundären Endpunkte genutzt. Die Biomarker (BM)-Population umfasst alle Patienten, bei denen Biomaterialien asserviert werden konnten.

Für die Fallzahlplanung werden als Standardgrößen angenommen: α -Fehler = 0,05; Power = 0,8; es ergibt sich ein $K = 7,85$ (Faktor, der sich aus α -Fehler und Power berechnet). Die folgende vereinfachte Formel wurde benutzt:

$$n = \frac{K [(R + 1) - p_2 (R^2 + 1)]}{p_2 (1 - R)^2}$$

n = benötigte Fallzahl

p1 = Ereignisrate in der Behandlungsgruppe (2 %)

p2 = Ereignisrate in der Kontrollgruppe (5 %)

R = relatives Risiko (p1/p2) = hier: 2 % / 5% = 0,4

Im Ergebnis werden 607 Patienten pro Gruppe benötigt. Die Fallzahlberechnung wird ggf. anhand der verblindet ermittelten Ereignisrate in der Studie angepasst.

Der primäre Endpunkt wird mit einem zwei-seitigen Signifikanz-Niveau von 5 % in der ITT-Population mit dem Fisher's Exact Test untersucht. Sekundäre Endpunkte werden mit demselben Test sequentiell getestet, bis eine Signifikanz auf dem 5%-Niveau nicht mehr erreicht wird. Danach sind diese exploratorisch und werden nur deskriptiv ausgewertet.

11 Anpassung der Stichprobengröße

Nach 500 abgeschlossenen Patienten wird die Frequenz des primären Endpunkts in den verblindeten Daten beurteilt. Es wird erwartet, dass dann ca. 37 Patienten eine Krankenhauspflege mit Sauerstoffpflicht erhalten haben. Gegebenenfalls erfolgt eine Anpassung der Stichprobengröße mit Erweiterung der Patientenzahl.

12 Datenmanagement, Datenschutz und Umgang mit Biomaterialien

Die medizinischen Daten inkl. klinischer Routine-Befunde werden in dieser Studie nicht pseudonymisiert. Es erfolgt jedoch eine Pseudonymisierung des Patienten für die Studie mit einer Studien-Patienten-ID, sobald die Daten zur Auswertung freigegeben werden. Die Zuordnung der Patientenklardaten mit der Studien-Patienten-ID verlässt dabei das Rekrutierungszentrum nicht. Das Rekrutierungszentrum wird vom Institut für Epidemiologie betrieben und beaufsichtigt, das vom Kompetenznetz Darmerkrankungen e.V. bei der Datenhaltung und durch die Gestellung geschulter Studienpersonals für die telefonische

Datenakquise unterstützt wird. Außerhalb des Rekrutierungszentrums wird ausschließlich mit Studien-Patienten-IDs umgegangen.

In einer für diese Studie eingerichteten, web-basierten Case Report Form (CRF)-Datenbank werden die in Abschnitt 19.3 des Anhangs aufgeführten Informationen gesammelt. Dabei erfolgt die Eingabe der Daten verblindet.

Die Daten der Serum- und Stuhl-Analysen werden im Institut für Klinische Molekularbiologie (Kiel) in einer digitalen Datenbank pseudonymisiert gespeichert. Die Datenspeicherung erfolgt selbstverständlich hinter der Firewall von CAU/UKSH und mit allen entsprechenden Sicherheitsvorkehrungen. Da das Projekt unter ärztlicher Leitung steht, unterliegen alle Mitarbeiter der ärztlichen Schweigepflicht. Eine Weitergabe der Daten an unberechtigte Dritte (insbesondere Arbeitgeber, Versicherungen) ist ausgeschlossen. Die Weitergabe von Proben und Informationen an wissenschaftliche Kooperationspartner erfolgt ausschließlich in pseudonymisierter Form, d.h. ohne Angaben zur Person. Bei Beendigung der Forschungsaktivitäten (frühestens nach 20 Jahren) werden die Proben und die dazu gehörenden Daten vernichtet. Auf Patientenwunsch erhalten diese eine Kopie mit ausführlicher Darstellung des Datenmanagements am Institut für Klinische Molekularbiologie. Diese entspricht den analytischen Standards, welche vom IHEC (International Human Epigenome Consortium; <http://ihec-epigenomes.org>) entwickelt wurden. Vor Analyse von Bioproben werden diese rekodiert und mit einem pseudonymisierten Datensatz verbunden.

Die Teilnahme an dieser klinischen Studie ist absolut freiwillig. Die Zustimmung kann jederzeit vom Patienten widerrufen werden. Daraus entstehen den Patienten keinerlei Nachteile. Gegebenenfalls bereits entnommene Proben werden dann unverzüglich vernichtet, und die Daten (außer bereits veröffentlichte/analytierte Daten) werden umgehend gelöscht, soweit technisch möglich (eine Löschung nach Auswertung ist nicht mehr möglich, darüber wird der Patient auch informiert). Einen etwaigen Widerruf der Zustimmung können die Patienten schriftlich an die Projektleitung der Klinik für Innere Medizin I, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Rosalind-Franklin-Str. 12, Haus K1, D-24105 Kiel, richten.

Es kann sein, dass im Rahmen dieses Forschungsvorhabens Patente entstehen. In diesem Fall besteht kein individueller Patentanspruch der Patienten basierend auf deren individuellen Daten oder individuellen biologischen Materialien.

13 Datenverifizierung

Da die Eingabe der Ergebnisse der Telefonkontakte direkt in die Datenbank erfolgt, findet kein systematisches Monitoring statt. Explorative Daten werden nur zentral erhoben und unterliegen den in Abschnitt 12 beschriebenen Datenverarbeitungsstandards am Institut für Klinische Molekularbiologie (Kiel). Falls erforderlich, werden zusätzliche Daten von den behandelnden Ärzten abgefordert.

14 Protokolländerungen nach Studienbeginn

Protokoll-Amendments

Jede Veränderung dieses Protokolls wird in einem Protokoll-Amendment dokumentiert, und benötigt die Zustimmung des Studienleiters. Diese Protokoll-Amendments werden den zuständigen Ethikkommissionen mitgeteilt und im Falle wesentlicher Änderungen vorher zur Genehmigung vorgelegt.

Protokollabweichungen

Abweichungen von diesem Protokoll sollen vermieden werden. Falls eine Protokollabweichung stattfindet, beauftragt der Studienleiter einen Datenabgleich, und die Folgen der Abweichung werden überprüft und diskutiert. Jede Abweichung wird im Studienordner dokumentiert.

Frühzeitige Beendigung der Studie

Der Studienleiter hat das Recht, die Studie zu jeder Zeit zu beenden. In diesem Fall muss der Studienleiter sicherstellen, dass die weiteren Entscheidungen dem bestmöglichen Wohl der Patienten entsprechen. Die Beendigung der Studie wird den zuständigen Ethikkommissionen mitgeteilt. Die Studie kann von einzelnen Studienzentren durch die verantwortlichen Kliniker beendet werden, falls

- die Fortführung der Studie aus ethischer oder medizinischer Sicht nicht zumutbar ist,
- die Ressourcen zur Fortführung der Studie nicht suffizient vorhanden sind, oder
- ein aussagekräftiges Resultat nicht als wahrscheinlich erscheint.

Einzelne Studienteilnehmer, dessen Gesundheit und Sicherheit gefährdet ist, können von beteiligten Klinikern nach deren Ermessen von der Studie ausgeschlossen werden.

15 Ethische Aspekte

Unabhängige Ethik-Kommission

Die unabhängige Ethik-Kommission der medizinischen Fakultät der CAU Kiel wird dieses Protokoll sowie die Probandeninformation und Probandeneinwilligung, deren Aktualisierungen (falls vorhanden) und jedes weitere den Patienten schriftlich ausgehändigte Material evaluieren.

End-of-Trial und End-of-Trial-Benachrichtigung

Das End-of-Trial ist definiert als Datum, an dem der letzte Studienteilnehmer an seiner letzten Studienvsiste telefonisch kontaktiert wird. Am Ende der Studie werden die zuständigen Ethik-Kommissionen hierüber benachrichtigt.

Ethische Durchführung der Studie

Diese klinische Studie wird gemäß ethischen Prinzipien in Anlehnung an die Deklaration von Helsinki und nach diesem genehmigten Protokoll durchgeführt.

16 Probandenversicherung

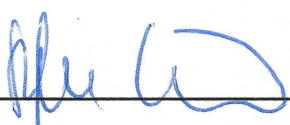
Für die Studie wird keine Probanden- oder Wegeunfallversicherung abgeschlossen. In der Patienteninformation wird ausdrücklich darauf hingewiesen, dass keine Probanden- oder Wegeunfallversicherung besteht.

17 Publikationsregeln

Nach Beendigung der Studie werden ein oder mehrere Manuskripte für gemeinsame Publikationen erstellt. Die Autorenschaft wird nach ICMJE-Kriterien vergeben (für eine offizielle Version siehe <http://www.ICMJE.org>). Die Autorenliste richtet sich nach den jeweiligen Richtlinien der entsprechenden Fachzeitschriften oder Kongressen. Auftragslabore haben bezüglich dieser Studie kein Publikationsrecht.

18 Unterschrift des Studienleiters

Kiel, 26.3.2020


Prof. Dr. med. Stefan Schreiber

19 Anhang

19.1 Patienteninformation

19.2 Aufbau der Online-Einwilligung

19.3 Case Report Form (CRF)

6. Statistical analysis plan for acute COVID-19

This section contains the statistical analysis plan for final analyses for acute COVID-19 in the final version 1.1 of 27 July 2022 (35 pp.).

STATISTICAL ANALYSIS PLAN

**Improvement of the nutritional status regarding nicotinamide
(vitamin B3) and the disease course of COVID-19**

COVit-2

Final analyses for acute COVID-19

Type of study: Interventional (Clinical Trial)

Biometrician: Regina Hollweck (Novustat)

Version number: 1.1

Date of issue: 27 July 2022

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Version History

SAP Version History Summary

| SAP Version | Date | Change | Rationale |
|-------------|---------------|---|--|
| 1 | 27 April 2022 | Not applicable | Initial release |
| 1.1 | 27 July 2022 | <ol style="list-style-type: none"> 1. Differentiation ITT and safety population 2. Primary analysis population RFITT 3. Subgroup analysis for complementary subgroups 4. Further specification of subgroups cardiovascular diseases 5. Definition of responders 6. Baseline description 7. Expanded description on missing data and collection of safety information 8. Duration of hospitalization as additional exploratory endpoint 9. Analyses of SF-36 and FACIT-F questionnaires for RFITT and RFPP with severe baseline scores and subscores (below or equal to the median) | <ol style="list-style-type: none"> 1. Safety analysis will include also patients without intake of study medication 2. More robust than RFPP 3. Complementary subgroups facilitate interpretation of results and are the gold standard for subgroup analyses 4. Patients with stroke show other behaviour in disease progress in newly released scientific publication 5. Responder analysis as additional exploratory analysis 6. Shortening of variable list for baseline description 7. Clarification of procedures 8. Important health economic endpoint 9. Focus on patients with moderate/severe baseline symptomatology. |

Signatures for Approval

27/07/22
(Date)


(Regina Hollweck, Trial Statistician)

02 Aug 2022
(Date)


(Prof. Dr. Stefan Schreiber, Principal Investigator)

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List of Abbreviations

| | |
|------------------|--|
| ACE2 | Angiotensin-converting enzyme 2 |
| AR(1) | Autoregression of lag 1 |
| B0AT1 | Sodium-dependent neutral amino acid transporter |
| CI | Confidence interval |
| CICR-NAM | Controlled-ileocolonic-release nicotinamide |
| DMB | Data Management Board |
| FACIT-F | Functional Assessment of Chronic Illness Therapy – Fatigue |
| ITT | Intention-to-treat |
| LS | Least square |
| M | Mean |
| MAR | Missing at random |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MMRM | Mixed model repeated measures |
| N | Number of observations |
| NAD | Nicotinamide adenine dinucleotide |
| NADP | Nicotinamide adenine dinucleotide phosphate |
| RFITT | Risk factor intention-to-treat |
| RFPP | Risk factor per protocol |
| SAP | Statistical Analysis Plan |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus type 2 |
| SD | Standard deviation |
| SF-36 | RAND 36-Item Health Survey |
| WHO | World Health Organisation |

Trial Summary

| | |
|-------------------------------|--|
| Principal Investigator | Prof. Dr. Stefan Schreiber and Prof. Dr. Matthias Laudes Department of Internal Medicine I University Medical Center Schleswig-Holstein (UKSH), Campus Kiel Arnold-Heller-Str. 3 24105 Kiel Telephone: +49 431 500-22201 Fax: +49 431 500-22204 E-mail: s.schreiber@mucosa.de |
| Sponsor | University Medical Center Schleswig-Holstein (UKSH), Campus Kiel Arnold-Heller-Str. 3 24105 Kiel |
| Title | Improvement of the nutritional status regarding nicotinamide (vitamin B3) and the disease course of COVID-19 |
| Protocol Title | Verbesserung des Ernährungsstatus bezüglich Nicotinamid (Vitamin B3) und Verlauf der COVID-19-Erkrankung |
| Acronym | COVit-2 |
| Background | Nicotinamide, a form of vitamin B3, plays an important role in chronic systemic inflammation and reduced circulating levels impede immune defense against coronaviruses. We also assume an anti-inflammatory barrier-strengthening property. Results from a pilot study suggest a potential efficacy of nicotinamide on the resolution of COVID-19 symptoms. Confirmatory data are needed to determine whether nicotinamide is effective in reducing the symptom burden in patients with COVID-19. |
| Study Design | Double-blind, placebo-controlled, randomized clinical trial. |
| Intervention Group | Nicotinamide, 1,000 mg/day p.o. (one each of 500-mg immediate-release and delayed-release nicotinamide tablets), for 4 weeks. |
| Control Group | Placebo, p.o. (2 tablets), for 4 weeks. |
| Randomization | Patients are randomized continuously in a 1:1 ratio according to a randomization scheme in which every second patient is |

| | |
|--|--|
| | randomized and the following one then receives the opposite allocation. |
| Blinding | Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) |
| Primary Analysis | Comparison of the change to baseline of the primary outcome between patients randomized to nicotinamide versus placebo using the Cochran-Mantel-Haenszel test for change of binary variables. Post hoc Fisher Exact test will be applied to compare the effect at week 2 (primary analysis time point). For the futility population, a total of 4 primary key symptoms will be analyzed using a hierarchical testing procedure. For continuous or ordinal scaled variables, a mixed effect model repeat measurement (MMRM) with fixed effect terms for baseline value, treatment group, time (categorical variables) and treatment-by-time interaction (metric or ordinal variables) will be used. |
| Primary Endpoint of the Acute Disease Phase | <p>Frequencies of key individual COVID-19 symptoms at week 0 (baseline), week 2 (primary time of analysis), week 4 and week 6:</p> <ol style="list-style-type: none"> 1. Performance drop (yes/no). <p>For the futility population, additional key symptoms will be analyzed as part of the primary endpoint:</p> <ol style="list-style-type: none"> 2. Ability to perform normal activities (quantified by the complaint scale for lower respiratory tract infections); 3. Cough (quantified by the complaint scale); 4. Fatigue (yes/no). |

1. Introduction

The present document aims at detailing the statistical analyses that will be performed for the study DRKS00021214 (NCT04751604) with the data from the acute disease phase of COVID-19 (week 0 to week 6) on the basis of the current study protocol (Version 2.3, dated 13 Dec 2021). This document has been written by the lead statistician and reviewed and agreed by the Principal Investigator of the trial.

The availability of tryptophan and its metabolites, especially nicotinamide (a form of vitamin B3) as a building block of NAD and NADP, are important factors in the immune system. Dysregulation can lead to immune deficiency, but also to chronic inflammation (1-5). Particularly in the context of pneumonia, certain metabolites of tryptophan are important and can strengthen the immune functions of the lungs and counteract chronic changes caused by inflammatory reactions in the lungs (6-12). Nicotinamide supplementation has a potent anti-inflammatory effect in both tryptophan-deficient and normally fed animals (13-16). Reduced levels of nicotinamide and thus NAD(P) also impede immune defense against coronaviruses (17) and cellular metabolism, especially in macrophages and epithelial cells. The latter in particular are subject to a critically high rate of renewal in the gut and lungs. Another link to SARS-CoV-2 beyond inflammation and the immune system may also be the SARS-CoV-2 entry receptor ACE2, as the presence of ACE2 on the cell surface allows uptake of tryptophan via the transporter B0AT1 (13). In severe courses of influenza, as in chronic inflammation, increased tryptophan degradation and thus increased kynurenine levels are observed, and inhibition of tryptophan degradation has beneficial effects in animal models (18, 19). In turn, there is evidence in a wide variety of virus types that nicotinamide can reduce viral replication and support the body's defense mechanisms, e.g., in the context of infections with vaccinia (20), human immunodeficiency virus (21), enteroviruses (21), or hepatitis B (22). Sufficient supply of B vitamins and particularly nicotinamide to strengthen the immune system is also recommended to combat SARS-CoV-2 infection (23-26). Here, the improvement of the defense against secondary bacterial infections in disease models is particularly emphasized (23). In the COVit trial, 1,000 mg of nicotinamide are administered, which is far below potentially harmful doses of several grams per day and very close to the acceptable daily intake of 900 mg/day (27, 28). Meanwhile, several publications suggest the use of nicotinamide in COVID-19 but there are no trial data available (17, 24-26, 29). In the pilot phase of the COVit trial, patients received 1,000 mg of conventional

nicotinamide or 245 mg of silica as an inert placebo-like dietary supplement. During a quality control of the data collection, it was surprisingly observed that significantly more patients in the nicotinamide group were already completely symptom-free after two weeks of administration than in the silica group. From the literature, depending on the patient population, very long convalescences can also be expected, e.g.

- 43% of patients were symptom-free at 14-21 days after positive SARS-CoV-2 test (30),
- <30% without respiratory symptoms after 1 month (31),
- 32% symptom-free at 30 days and still only 34% at 60 days after SARS-CoV-2 diagnosis (32), or
- only 12.6% symptom-free after a mean of 60 days (33).

Moreover, COVID-19 has been shown to be a systemic disease with an unexpectedly high frequency of gastrointestinal symptoms (34). It has been published that the gut microbiome can be significantly and, in some cases, negatively affected by COVID-19 (35). The link between the gut microbiome and the metabolism of tryptophan and nicotinamide plays an important role in intestinal inflammation (13-16). Therefore, two different tablets, each containing 500 mg nicotinamide, are now being used as investigational drugs in the main phase of the COVIt trial: the conventional immediate-release nicotinamide tablets from the pilot phase and the newly developed controlled-ileocolonic-release nicotinamide (CICR-NAM) tablets, which release nicotinamide in a delayed and continuous manner starting in the lower small intestine. This should increase the gastrointestinal beneficial effects of nicotinamide, including those specifically on the microbiome and its interaction with the intestine, as conventional nicotinamide is absorbed very rapidly into the circulation (36). A study with a CICR-NAM prototype has shown that systemic exposure from this new dosage form will be lower than in the pilot phase of the study (36), in which exposure was already close to the acceptable daily intake anyway (see above). This will further reduce the already minimal risk of side effects.

2. Study Objectives

2.1. Primary Objective of the Main Study

The primary objective of the main study is to investigate the hypothesis that COVID-19 patients lose individual COVID-19 symptoms more quickly in an outpatient setting when supplemented with 1,000 mg of nicotinamide.

2.2. Secondary Objectives

The secondary objectives of the main study are to examine the following parameters: severity of individual symptoms, complete resolution of symptoms after 2, 4 or 6 weeks and the time from diagnosis to freedom from individual or all symptoms.

2.3. Exploratory Objectives

The WHO COVID-19 Ordinal Scale for Clinical Improvement (37) and the frequency of severe COVID-19 (examination in an emergency department, hospitalization with a continuous oxygen requirement of at least 24 hours, intensive care requirement, ventilation requirement or death by COVID-19) will be investigated in an exploratory fashion.

The findings from the pilot trial (COVit-1), in which only immediate-release nicotinamide was supplemented, and from the COVit-2 trial will be compared.

Changes in fatigue and quality of life will be assessed using validated questionnaires (FACIT-F and SF-36).

Further exploratory analyses on up to 20 selected patients will measure blood count, standard blood profile, various inflammatory markers and the metabolome in the blood, in particular tryptophan metabolism. In these patients, the virus strain will also be determined by sequencing from nasopharyngeal swabs. In selected patients, a detailed pharmacokinetic analysis can be performed. In the stool, changes in the microbiome (in 100-300 patients) as well as in the metagenome and metabolome (in a subgroup) will be analyzed.

3. Trial Methods

3.1. Trial Design

This is a double-blinded, randomized, placebo-controlled parallel group trial.

3.2. Randomization and Blinding

Patients are randomized in one of both treatment groups (nicotinamide or placebo) continuously as they are enrolled. The study is blinded for participants, care providers, investigators and outcome assessors as well as for the statisticians. The staff distributing the trial supplements is not blinded to ensure proper delivery. Trial supplements are provided in neutral containers. The blinding will not be violated by interim analyses as the allocation of randomization codes to treatment groups is not broken.

3.3. Sample Size

The data available at the beginning of the COVIt-2 trial suggested that at most 40% of the already symptomatic COVID-19 patients would be completely symptom-free two weeks after the start of the intervention (primary endpoint defined at that time). This would likely have been the case in the placebo-treated patient population. The hypothesis was that this 40% event rate could be increased to 50% with the use of nicotinamide. Therefore, the following default values were assumed in the primary sample size calculation: α -error = 0.05; power = 0.8; the result is $K = 7.85$ (factor calculated from α -error and power).

The following simplified formula was used:

$$n = \frac{K [(R + 1) - p_2 (R^2 + 1)]}{p_2 (1 - R)^2}$$

n = required number of cases

p_1 = event rate in the treatment group (50%)

p_2 = event rate in the control group (40%)

R = relative risk (p_1/p_2) = here: 50% / 40% = 1.25

As a result, 385 patients were needed per group. With an assumed drop-out rate of close to 10%, 420 patients per group were to be included. Based on the results of the futility analysis after $n=400$ patients, the Data Management Board (DMB) of the COVIt-2 trial recommended to keep the previously planned sample size of approximately 840 patients, to focus the primary endpoint on key symptoms (section 6.1.1.) and to select the subgroup of patients with at least one risk factor for severe COVID-19 as the primary clinical analysis population. To ensure that the subpopulation recruited after the futility analysis also included an adequate proportion of such patients, the frequencies of risk factors were monitored in a blinded manner and 900 patients were recruited as a safeguard against possibly increased dropout rates.

3.4. Framework

This clinical investigation evaluates the efficacy of a 4-week dietary intervention with a combination of 500 mg immediate-release nicotinamide and 500 mg controlled-ileocolonic release nicotinamide (CICR-NAM) on the recovery from COVID-19 symptoms both in a short-term (up 6 weeks) and a long-term setting (at approximately 6 months). The present SAP describes the analyses pertaining to the short-term setting.

3.5. Investigational Product

The trial uses four investigational products: conventional tablets that start to release nicotinamide immediately after reaching the stomach (immediate-release formulation) as well as novel tablets for CICR-NAM plus a similarly constructed matching placebo. The reasons for applying the innovative tablets for release in the ileum and colon are the gastrointestinal infections with SARS-CoV-2 and recent data showing dysbalances in the intestinal microbiome of COVID-19 patients. CICR-NAM was designed to beneficially influence the intestinal microbiome by topically increasing nicotinamide availability.

3.5.1. Nicotinamide Group

Participants assigned to the nicotinamide group self-administer 2 nicotinamide tablets (1 conventional 500-mg tablet and 1 500-mg tablet with CICR-NAM) for 4 weeks. The overall dose strength of the verum tablets is 1,000 mg nicotinamide, taken once daily with breakfast.

3.5.2. Control Group

Participants randomized to the control group self-administer 2 placebo tablets with an appearance corresponding to the respective nicotinamide tablets in the same manner as described above for the nicotinamide group.

3.6. Study Duration and Schedule

The study duration for the individual patient (see schedule below) comprises the 4-week active treatment period, a safety follow-up at 6 weeks and a recall at 6 months with characterization for remaining COVID-19 symptoms (post-COVID syndrome, PCS). Recruitment started on 01 February 2021 and the last patient was randomized on 17 January 2022. Week 6 observations were completed in March 2022. It is expected that the last data of the 6-month follow-up will be obtained in late July 2022. A planned futility analysis was performed in November 2021 after the 6-week data for 400 patients were available (see study protocol version 2.2 and SAP Futility Analysis, version 1.0).

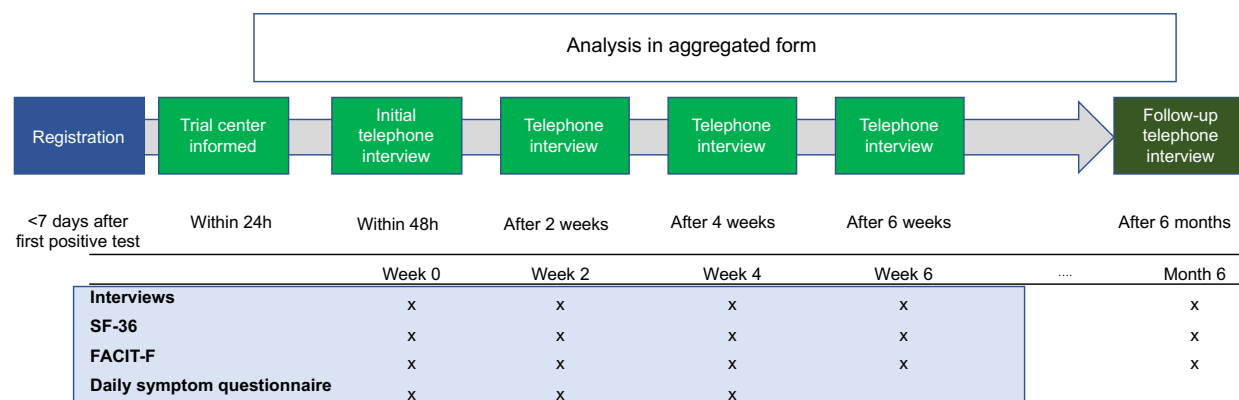


Figure 1: Scheme of visits during the trial. The present SAP describes the analyses until Week 6 (light blue box).

4. STATISTICAL PRINCIPLES

4.1. Confidence Intervals and P-Values

For all analyses, two-sided 95% confidence intervals (CI) will be reported. Confidence intervals provide an adequately plausible range for the true value related to the measurement of the point estimate. Statements are possible on the direction of the effects, as well as its strength and the presence of a statistically significant result. For (approximately) normally distributed variables, parametric 95% CI will be shown. If this assumption is violated, the corresponding CI will be estimated by bootstrapping methods.

In contrast to confidence intervals, p-values give the difference from a previously specified statistical level α . P-values will be calculated in case of 1) stated hypotheses or 2) in an exploratory fashion to evaluate the difference from a statistical level α . P-values will be shown with three decimal places, whereby p-values less than .05 will be considered as statistically significant. If not otherwise stated, p-values are two-tailed.

4.2. Adherence and Protocol Deviations

The planned analysis will be performed according to the clinical investigation plan, its amendments, and this statistical analysis plan. If there are contradictions between the clinical investigation plan or its amendments and this statistical analysis plan, this analysis plan will prevail.

If a protocol deviation or concurrent illness / adverse event occurs, which, according to the clinical judgment of the Investigator, may invalidate the study by pharmacokinetic or pharmacodynamic interference with the trial products, the subject will be withdrawn by the Investigator.

4.3. Analysis Populations

The safety population will include all randomized subjects.

The intention-to-treat (**ITT**) population will include all randomized subjects who have received at least one dose of any study treatment. ITT has been carried forward for understanding the interaction between nicotinamide and immune reactions to SARS-CoV-2.

According to the recommendations of the DMB after the planned futility analysis, the primary analysis population for efficacy will be the **RFITT** population. The RFITT population will be defined as all subjects of the ITT population with a least one characteristic or underlying medical condition associated with an increased risk of developing severe illness from COVID-19, selected from the group consisting of

- an age of ≥ 60 years;
- a body mass index of ≥ 30.0 ;
- type 1 diabetes;
- type 2 diabetes;
- cardiovascular diseases;
- high blood pressure;
- stroke;
- asthma;
- chronic obstructive pulmonary disease;
- other chronic lung diseases;
- current or former smokers (the latter being defined as patients who smoked more than 100 cigarettes or other smoking products in total so far, but have not smoked for at least 4 weeks);
- chronic liver diseases;
- chronic kidney diseases;
- cancer;
- organ transplants;
- current immunosuppressive therapy;
- chronic neurological diseases (multiple sclerosis, Parkinson's disease);

The **RFPP** population will be defined as all subjects of the RFITT population, excluding those patients who

- drop out or
- do not comply regarding investigational product intake for at least 80%, *i.e.* intake of investigational product for at least 11 of 14 days between each study interval: week 0 – week 2 and week 2 – week 4.

Futility analysis was performed after approximately 50% of patients had completed the Week 6 interview. All of these patients (n = 402) form a separate futility subgroup.

5. TRIAL POPULATION

5.1. Eligibility

5.1.1. Inclusion Criteria

- SARS-CoV-2 infection confirmed by laboratory findings; the positive test must not date back more than 7 days.
- Relevant symptoms of a SARS-CoV-2 infection, *e.g.* in the respiratory or gastrointestinal tract.
- The patient has been able to give written consent via a website before any trial procedure is performed and can comply with the trial-dependent prerequisites and requirements.
- The patient is 18 years or older.

5.1.2. Exclusion Criteria

Exclusion criteria are vaccination against SARS-CoV-2 before inclusion, current participation in another study and pregnancy or breast-feeding.

5.2. Recruitment

A flow diagram showing the progress through the phases of the trial (enrolment, intervention allocation, follow-up, and data analysis) will be produced for both treatment groups.

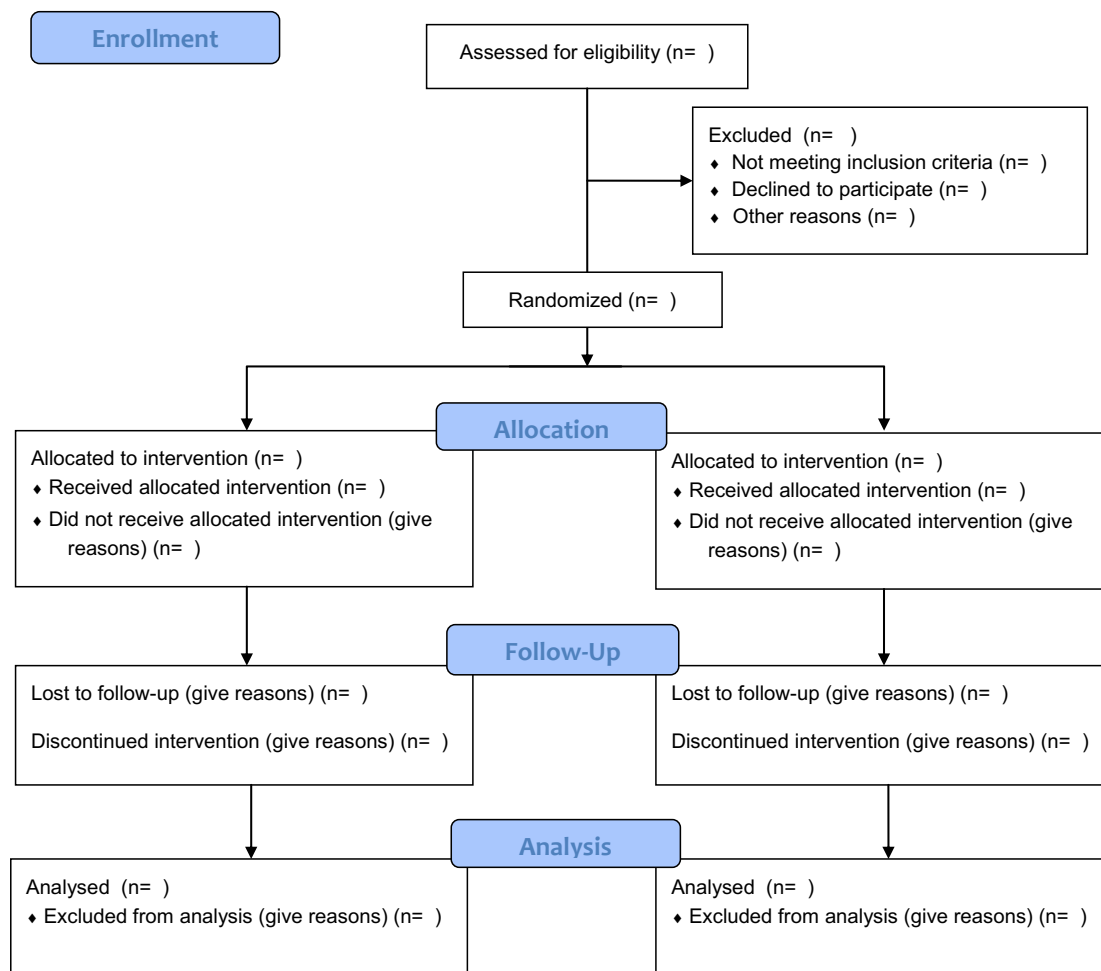


Figure 2: Flow chart according to CONSORT requirements.

5.3. Withdrawal/Follow-up

All subjects with evaluable data will be used for each analysis, irrespective of whether or not the subject subsequently dropped out.

The number of drop-outs and withdrawals will be reported as well as the time spent on the study (in days since enrolment) for those participants not completing the study protocol until the 6-weeks period.

6. ANALYSIS

6.1. Outcome Definition

6.1.1. Primary Outcomes

Frequencies of key individual COVID-19 symptoms at week 0, week 2 (primary time of analysis), week 4 and week 6:

1. Performance drop (yes/no).

For the futility population, additional key symptoms will be analyzed as part of the primary endpoint:

2. Ability to perform normal activities (quantified by the complaint scale for lower respiratory tract infections);
3. Cough (quantified by the complaint scale);
4. Fatigue (yes/no).

6.1.2. Secondary Outcomes

Interviews:

- Frequencies of individual COVID-19 symptoms (all symptoms recorded and not part of the primary endpoint) at week 0, week 2 (primary time of analysis), week 4 and week 6.

In the primary analysis population RFITT, the key secondary outcomes *ability to perform normal activities (quantified by the complaint scale)* and *cough (quantified by the complaint scale)* in patients with severe complaints indicated by a complaint scale score of >3, respectively, as well as *fatigue (yes/no)* will be analyzed first and tested hierarchically. The complaint scale for lower respiratory tract infections has the following gradations: 0 = normal, 1 = very minor problem, 2 = minor problem, 3 = moderately poor, 4 = poor, 5 = very poor, 6 = maximally poor.

- Severity of all individual COVID-19 symptoms (all symptoms recorded and not part of the primary endpoint, including interviews and daily symptom questionnaire) at week 0, week 2 (primary time of analysis), week 4 and week 6 or on a daily basis, respectively.
- Frequency of being completely symptom-free after 2 weeks.
- Frequency of being completely symptom-free after 4 weeks.
- Frequency of being completely symptom-free after 6 weeks.
- Time from diagnosis to resolution of individual symptoms (in 2-week intervals, up to 6 weeks after the beginning of the dietary intervention).
- Time from diagnosis to complete resolution of symptoms (in days and in 2-week intervals, up to 6 weeks after the beginning of the dietary intervention).

Daily symptom questionnaire:

- Time from diagnosis to resolution of individual symptoms (in days, up to day 28 of the dietary intervention, *i.e.* up to approximately 35 days).

- Time from diagnosis to complete resolution of symptoms (in days, up to day 28 of the dietary intervention).

6.1.3. Exploratory Outcomes

Exploratory analyses will assess dose and time effects by using appropriate statistical models. Especially, analyses in the time course will adjust for the fact (by, e. g., mixed models or covariance pattern analyses) that measurements are repeatedly taken within the same subject. Various exploratory analyses are planned:

- World Health Organization (WHO) COVID-19 Ordinal Scale for Clinical Improvement (see <https://www.who.int/publications/i/item/covid-19-therapeutic-trial-synopsis>) at week 0 (baseline), week 2, week 4 and week 6.
- Severe COVID-19, defined as occurrence of one of the following characteristics:
 - a. Examination in an emergency department;
 - b. Hospitalization with a continuous oxygen requirement of at least 24 hours;
 - c. Intensive care requirement;
 - d. Ventilation requirement;
 - e. Death by COVID-19.
- Duration of hospitalization in days.
- Comparison between the findings from the pilot trial (COVit-1), in which only immediate-release nicotinamide was supplemented, and from the COVit-2 trial.
- Change to Baseline of the 40-item Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F) questionnaire and its subscales at week 2, week 4 and week 6.
- Change to Baseline of the SF-36 questionnaire (RAND 36-Item Health Survey 1.0) and its subscales at week 2, week 4 and week 6.
- Changes in blood levels of tryptophan (in selected patients).
- Changes in blood levels of tryptophan metabolites (in selected patients).
- Change in blood levels of inflammatory markers (C-reactive protein, interleukin-6, ferritin, neopterin, D-dimers) (in selected patients).
- Changes in blood count and standard blood profile (in selected patients).
- Changes in blood metabolome composition (in selected patients).
- Strain of SARS-CoV-2 virus (in selected patients).
- Changes in stool microbiome composition (in selected patients).

- Changes in stool metagenome composition (in selected patients).
- Changes in stool metabolome composition (in selected patients).
- Pharmacokinetics (in selected patients).

6.2. Baseline Patient Characteristics

Demographic characteristics will include

- Age
- Gender
- Height
- Weight
- Ethnicity

Demographic and Baseline characteristics will be summarized descriptively by treatment group for the ITT, RFITT and RFPP analysis set. Continuous variables will be summarized by number of subjects, mean (M), standard deviation (SD), median, minimum, and maximum values. Categorical variables will be summarized by number and percentage of subjects in each category. Comparability of treatment groups will be assessed based on the review of statistical summaries, such as mean/median or percentages in each category, among baseline characteristics and statistical testing (Chi-Square/Fisher Exact test for categorical variables, t-Test or Mann-Whitney-U test for metric variables).

6.3. Prior/Concomitant Medication and Supplementation

Counting rules for prior, concomitant, and post-treatment medications or dietary supplements: prior medication/supplementation refers to all medications/supplements that were taken prior to randomization. Concomitant medication/supplementation refers to all medications taken during the double-blind trial supplementation period (*i.e.*, the duration when the medication/supplementation is taken overlaps the double-blind trial supplementation period at any single time), including those continued from pre-treatment. Post-treatment medications/supplements include medications/supplements that were started after the end of the double-blind trial supplementation period.

7. ANALYSIS METHODS

7.1. Pre-Processing

7.1.1. Interview Data

Differences between week 2, week 4 and week 6 to baseline (week 0) will be calculated as: week 2 – week 0, week 4 – week 0 and week 6 – week 0, respectively. For ordinal variables (e.g., symptoms quantified by the complaint scale), the difference is a metric variable. For binary variables [symptom present (yes/no)], the change to baseline will be divided into the categories worsening (symptom not present at week 0 but at week x – relevant for safety / ITT population), persistence (symptom present at baseline and still present at week x) or resolution (symptom present at baseline and absent at week x).

The event „symptom-free after x weeks“ will be calculated separately for all recorded symptoms. Patients who show no symptom at week x will be considered as symptom-free after x weeks, even if new onsets of symptoms are recorded at later time points. Subsequently occurring new symptoms may be due to causes other than acute COVID-19 and are therefore not taken into account.

Time from baseline to resolution of individual symptoms will be calculated as difference between visit date (week) at the event symptom-free and baseline visit (week 0) in weeks.

Time from baseline to complete resolution of symptoms will be calculated as difference between the date of symptom resolution (as stated by the patient in the following interview) and the baseline visit (week 0) in days. If the exact date could not be given by the patients, the week or month will be recorded. Following a worst case scenario, the last day of the week or month will be imputed to calculate the difference to baseline visit.

For patients who did not reach the status symptom-free within the trial period, the time from baseline to resolution of individual symptoms is calculated as difference between the follow-up visit to baseline (maximal observation time for patients with censored events).

7.1.2. Daily Symptom Questionnaire

The event „symptom-free after x days“ will be calculated separately for all recorded symptoms of the daily symptom questionnaire. Patients who show no symptom at day x will be considered as symptom-free after x days. Time from baseline to resolution of individual or all symptoms will be obtained directly from the diary.

7.1.3. FACIT-F

For the FACIT-F questionnaire, the subscales physical well-being, social/familiar well-being, emotional well-being, functional well-being, fatigue subscale, FACIT-F TOI, FACIT-G total score and FACIT-F total score will be calculated after data cleaning within an external Excel scoring sheet as described in the manual (39).

7.1.4. SF-36

The scoring of the RAND 36-Item Health Survey will be performed after double data entry and cleaning using an external scoring scheme in MS Excel. Scale and subscales will be calculated and sent for data analyses. Items that are left blank (missing data) are not taken into account when calculating the scale scores. Hence, scale scores represent the average for all items in the scale that the respondent answered. Values of the reference population are given in Table 1 (40).

Table 1: Reference values for SF-36 supplied by the RAND corporation based on the Medical Outcomes Study (n=2471).

| Scale | Items | Alpha | Mean | SD |
|----------------------------|-------|-------|-------|-------|
| Physical functioning | 10 | 0.93 | 70.61 | 27.42 |
| Role functioning/physical | 4 | 0.84 | 52.97 | 40.78 |
| Role functioning/emotional | 3 | 0.83 | 65.78 | 40.71 |
| Energy/fatigue | 4 | 0.86 | 52.15 | 22.39 |
| Emotional well-being | 5 | 0.90 | 70.38 | 21.97 |
| Social functioning | 2 | 0.85 | 78.77 | 25.43 |
| Pain | 2 | 0.78 | 70.77 | 25.46 |
| General health | 5 | 0.78 | 56.99 | 21.11 |
| Health change | 1 | — | 59.14 | 23.12 |

7.2. Main Analysis

The standard summary statistics for continuous baseline and outcome variables will be: N, mean, standard deviation, 95% CI, median, minimum and maximum. The standard summary statistics for categorical baseline and outcome variables will be absolute and relative frequencies (expressed as percentage).

Prior to analysis, all metric variables will be checked with respect of the normality assumption to draw reliable interpretations and conclusions of the research. Skewness and kurtosis will be used to assess the distribution form. If the absolute

values are less than 1, the deviation from the normal distribution will be considered safe (41).

Analyses of primary and secondary endpoints will be conducted on the ITT Analysis Set and for the RFITT and RFPP populations. The primary analysis population for efficacy will be RFITT. Change from baseline values (difference between measurements at week x and week 0) will be analyzed for all continuous efficacy variables at each time point collected.

The primary efficacy variable symptom (yes/no) will be analyzed using change to baseline, considered as worsening (symptom present at week x but not at baseline; relevant for safety / ITT population), persistence (no change) or resolution (symptom present at baseline but not at week x). The frequency of patients with symptom worsening, persistence or resolution for all weeks by treatment group will be compared using the Cochran-Mantel-Haenszel test. Post-hoc tests for each week will be calculated using Fisher Exact test with Benjamini & Hochberg (56) adjustment for multiple testing. The Woolfe test will be performed to test for homogeneity of odds ratios across time. In case of significant p-values of the Woolfe test, the Cochran-Mantel-Haenszel test would not be appropriate. In this case, Fisher Exact tests for each timepoint will be used instead of the Cochran-Mantel-Haenszel test.

For evaluation of the four ordered primary endpoints of the futility population, the unadjusted p-values from the Fisher Exact test of the change from baseline to week 2 between the treatment groups will be extracted. These p-values will be used for the hierarchical testing procedure using a fallback method.

The two metric/ordinal primary key symptoms “ability to perform normal activities” and “cough” (measured on a scale) will be analyzed using a mixed effect model repeat measurement (MMRM) approach with baseline value as a covariate, fixed effect terms in the model group, week, and group-by-week interaction, and an unstructured covariance matrix. Using this model, the difference in change from baseline between the groups will be estimated based on Least Square (LS) mean difference with corresponding 95% CI and p-value. In the event that the unstructured covariance matrix is non-estimable (due to lack of convergence) when fitting the MMRM to the observed study data, an autoregression of lag 1 (AR(1)) covariance matrix will be used instead. For the comparison of selected visits, contrasts will be used. The treatment effect will be evaluated using the contrast treatment group (A vs. B) of change from week 2 to week 0 (baseline). Other contrasts for other time points will be also reported,

but not used for evaluation of the primary study endpoint. Hierarchical multiple testing with fallback procedure will be applied to the unadjusted contrast of treatment group (A vs. B) for the change from week 2 to week 0 (baseline) (see section 7.6).

Other symptoms recorded at the interviews will be analyzed by the same models in an exploratively manner only. Continuous or ordinal secondary endpoints (severity of symptoms) measured at different time points will be analyzed using the same MMRM approach as used for the metric primary efficacy variables. For categorical secondary endpoints [other symptoms (yes/no) recorded within the interviews], Cochran-Mantel-Haenszel and post-hoc Fisher Exact tests for change of binary variables (symptom worsening, persistence or resolution) will be applied.

For every symptom in the efficacy analyses in the RFITT population, only patients suffering from the respective symptom at Baseline will be included into the analysis. For continuous or ordinal endpoints (e.g., severity of symptoms), further subgroups may be formed according to levels of intensity or severity (e.g., patients with at least moderate symptoms or patients with severe symptoms). The first level of subgroup analysis will focus on patients with substantial symptomatology. For example, the key outcomes *ability to perform normal activities (quantified by the complaint scale)* and *cough (quantified by the complaint scale)* will first be analyzed in patients with severe complaints indicated by a complaint scale score of >3, respectively (see section 6.1.2.), and analyses of the FACIT-F and SF-36 questionnaires will focus on patients with baseline outcome (scales and subscales) below or equal to the corresponding median of the RFITT population.

For time-to-event analyses, a Kaplan-Meier approach will be used. Log rank test will be performed to test if the time to event differs between treatment groups. A Cox-proportional hazards Model will be used to evaluate and estimate the impact of the treatment group. Time-dependent covariates may be included if necessary. For interviews, time is recorded in 2-week intervals. In the symptom questionnaires, symptoms are recorded daily during the dietary supplementation of 28 days.

For the patient questionnaires (FACIT-F and SF-36), data reliability will be assessed through internal consistency using Cronbach's alpha coefficient ($\alpha \geq 0.80$) and item to total correlation of more than 0.20. The average variance extracted will be calculated to assess discriminant validity.

For analysis of the safety of the supplementation, all symptoms reported during the course of the study which were not present at baseline or increased in severity will be

MedDRA®-coded, listed by system organ class and preferred term, and their frequency will be compared between the two groups using Chi-square or Fisher Exact test.

All statistical tests will be 2-tailed with a type I error of .05 unless otherwise stated.

7.3. Subgroups

Subgroups analyses will be performed on demand in order to further define responders. Possible subgroups might be defined in accordance with the futility analysis:

Risk groups as subgroups of the RFITT populations:

- age of ≥ 60 years;
- body mass index of ≥ 30.0 or type 2 diabetes;
- cardiovascular diseases;
- high blood pressure;
- stroke;
- asthma, chronic obstructive pulmonary disease or other chronic lung diseases;
- current or former smokers (the latter being defined as patients who smoked more than 100 cigarettes or other smoking products in total so far, but have not smoked for at least 4 weeks)

Further subgroups might be defined based on biomarkers.

Within subgroup analyses also complementary subgroups will be analyzed.

Responders to primary and key secondary endpoints will be considered as additional population/subgroups.

Responders are defined as patients of ITT or RFITT or RFPP who show improvement from week 2 to week 6 compared to baseline in the primary and/or at least one of the secondary key endpoints. Improvement will be considered as resolution of symptom (binary endpoint) or reduction of severity of at least 3 scalepoints (scale).

Subgroup analyses will be performed using methods described in section 7.2.

7.4. Final Analyses and Reporting

The final analyses described in this SAP will be performed after the database has been cleaned and locked for the data up to week 6. The final report will include all analyses including up to week 6.

7.5. Missing Data

All effort will be made to achieve complete capture of all data from all patients, including those patients who discontinue treatment. Data of primary and secondary endpoints will be captured by interviews and the interviewers are instructed to contact patients multiple times until they receive feedback from them. The frequency of missing data will be checked with respect to treatment differences in advance.

Within the dataset, symptoms are reported only in case they are present and could be observed. So, all patients with no reported symptoms and thus missing values are treated as patients with no symptoms.

Statistical methods that do not employ imputation like MMRM or Chi-Square Test will be used for analyses. The use of the MMRM model assumes implicitly that data are missing at random (MAR).

7.6. Multiple Testing Procedure

For testing several symptoms, hierarchical testing with a fallback procedure will be applied. For the primary endpoints of the futility population, the type I error rate (α) is partitioned among four hypotheses of interest. Testing hypotheses proceeds in the following order:

1. Performance drop (yes/no);
2. Ability to perform normal activities (quantified by the complaint scale);
3. Cough (quantified by the complaint scale);
4. Fatigue (yes/no).

As long as hypotheses are rejected, the Type I error rate can be accumulated, making tests of later hypotheses more powerful than under the Bonferroni procedure. Unlike the fixed sequence test, the fallback test allows consideration of all hypotheses even if one or more hypotheses are not rejected early in the process, thereby avoiding a common concern about the fixed sequence procedure.

The Type I error rate (α) is divided in the following proportions:

1. Performance drop (yes/no): 60% (.03);
2. Ability to perform normal activities (quantified by the complaint scale): 15% (.0075);
3. Cough (quantified by the complaint scale): 15% (.0075);
4. Fatigue (yes/no): 10% (.005).

In the primary analysis population RFITT, sequential hierarchical testing (α level .05) is applied to the three key secondary endpoints:

1. Ability to perform normal activities (quantified by the complaint scale);
2. Cough (quantified by the complaint scale);
3. Fatigue (yes/no).

7.7. Statistical Software

R version 3.6.3 using RStudio Version 1.1.463 or a more recent version at the time of the analysis will be used for all statistical analyses.

8. Safety

In the COVit trial, 1,000 mg of nicotinamide are administered, which is far below potentially harmful doses of several grams per day and very close to the acceptable daily intake of 900 mg/day (see section 1). Moreover, half of the dose is administered in a formulation targeting the ileum and colon, which additionally reduces the pulse of systemic exposure from immediate-release (conventional) nicotinamide that was considered when defining the acceptable daily intake (see section 1). At higher doses than those used in the COVit trial, nicotinamide can cause heartburn, nausea, diarrhoea and other gastrointestinal symptoms. Cases of liver toxicity were usually observed only after longer exposure to at least 3,000 mg of systemically available nicotinamide per day. Adverse events and possible side effects can easily be monitored and will be recorded in the symptom questionnaire of the trial, which therefore also serves a pharmacovigilance-like purpose far exceeding the usual practice with the administration of dietary supplements.

In addition to the general surveillance of adverse events and safety aspects described in section 7.2., frequencies of symptoms recorded in the patient interviews will be specifically used to investigate possible side effects during the course of the study at week 2 to week 6 (if these were not present already at week 0). Therefore, all symptoms recorded in the patient interviews (queried as present/absent or via a scale of complaints, or noted as further symptoms) will be reported. Adverse events are defined as symptoms that were not present at baseline but were recorded at week 2 to 6 (binary variables and free text notation) or showed an increase in a scale of complaints of at least one point compared to baseline or to the previous visit during the course of the trial.

Adverse events will be MedDRA[®]-coded and reported in tabulated form including system organ class, preferred term, description of the adverse event/complaint, group

allocation, number and frequency (in %) of affected individuals as well as the total number of events. The Fisher Exact Test will be used to compare the frequency of adverse events between treatment groups.

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Appendix A: Summary of Analyses

| Variable | Outcome | Population ^a | Statistical Method | Type |
|---|---|--------------------------|--|--------------|
| Frequency of individual COVID-19 symptoms at week 2 (primary time point), week 4 and week 6 compared to baseline (week 0) – worsening, persistence and resolution | Categorical (worsening / persistence / resolution) | ITT | Cochran-Mantel-Haenszel test, post-hoc Fisher Exact test | Longitudinal |
| Frequency of individual COVID-19 symptoms at week 2 (primary time point), week 4 and week 6 compared to baseline (week 0) – persistence and resolution | Binary (persistence / resolution) | ITT, RFITT , RFPP | Cochran-Mantel-Haenszel test, post-hoc Fisher Exact test | Longitudinal |
| Change of the severity of individual COVID-19 symptoms from week 2 (primary time point), week 4 and week 6 to baseline (week 0) | Metric | ITT, RFITT , RFPP | MMRM | Longitudinal |
| Frequency of being completely symptom-free after 2 weeks | Binary (yes/no) | ITT, RFITT , RFPP | Chi-Square test or Fisher Exact test | Longitudinal |
| Frequency of being completely symptom-free after 4 weeks | Binary (yes/no) | ITT, RFITT , RFPP | Chi-Square test or Fisher Exact test | Longitudinal |
| Frequency of being completely symptom-free after 6 weeks | Binary (yes/no) | ITT, RFITT , RFPP | Chi-Square test or Fisher Exact test | Longitudinal |
| Time from diagnosis to resolution of individual symptoms (in 2-week intervals, up to 6 weeks) | Time to event | ITT, RFITT , RFPP | Kaplan-Meier, Cox-PH | Longitudinal |
| Time from diagnosis to complete resolution of symptoms (in 2-week intervals, up to 6 weeks) | Time to event | ITT, RFITT , RFPP | Kaplan-Meier, Cox-PH | Longitudinal |
| Time from diagnosis to resolution of individual symptoms (in days, up to day 28 of the dietary intervention) as reported in the daily symptom questionnaire | Time to event | ITT, RFITT , RFPP | Kaplan-Meier, Cox-PH | Longitudinal |
| Time from diagnosis to complete resolution of symptoms (in days, up to day 28 of the dietary intervention) as reported in the daily symptom questionnaire | Time to event | ITT, RFITT , RFPP | Kaplan-Meier, Cox-PH | Longitudinal |
| Time from diagnosis to complete resolution of symptoms (in days, up to 6 weeks) as reported in patient interviews | Time to event | ITT, RFITT , RFPP | Kaplan-Meier, Cox-PH | Longitudinal |

| | | | | |
|---|--------|--------------------------|------|--------------|
| Change of scale and subscales of FACIT-F from week 2 (primary time point), week 4 and week 6 to baseline (week 0) | Metric | ITT, RFITT , RFPP | MMRM | Longitudinal |
| Change of scale and subscales of SF-36 from week 2 (primary time point), week 4 and week 6 to baseline (week 0) | Metric | ITT, RFITT , RFPP | MMRM | Longitudinal |

Annotation: a) Primary analysis population: RFITT

7. Statistical analysis plan for the 6-month follow-up

This section contains the statistical analysis plan for the analyses at the 6-month follow-up in the final version 1.0 of 24 October 2022 (47 pp.).

STATISTICAL ANALYSIS PLAN

Improvement of the nutritional status regarding nicotinamide (vitamin B3) and the disease course of COVID-19

COVit-2

Follow-up at 6 months after COVID-19

Type of study: Interventional (Clinical Trial)

Biometrician: Regina Hollweck (Novustat)

Version number: 1.0

Date of issue: 24 October 2022

Type of report: Follow-up at 6 months after COVID-19



SAP Version History Summary

| SAP Version | Date | Change | Rationale |
|-------------|-----------------|----------------|-----------------|
| 1 | 24 October 2022 | Not applicable | Initial release |
| | | | |
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| | | | |
| | | | |

Signature

24. Oct 22
(Date)


(Regina Hollweck, Trial Statistician)

27.10.2022
(Date)

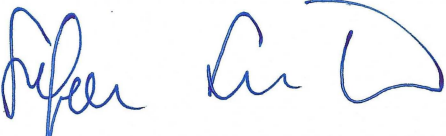

(Prof. Dr. Dr. h.c. Stefan Schreiber, Principal Investigator)

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List of Abbreviations

| | |
|------------------|--|
| BRS | Brief Resilience Scale |
| CI | Confidence Interval |
| CICR-NAM | Controlled Ileocolonic Release of Nicotinamide |
| COVID-19 | Coronavirus Disease 2019 |
| DMB | Data Management Board |
| FACIT-F | Functional Assessment of Chronic Illness Therapy – Fatigue |
| GAD-7 | Generalized Anxiety Disorder 7 |
| ITT | Intention-to-Treat |
| LOESS | Locally-weighted Scatterplot Smoothing |
| M | Mean |
| MDP | Multidimensional Dyspnoea Profile |
| MFI | Multidimensional Fatigue Inventory |
| MMRM | Mixed Model Repeated Measures |
| N | Number of Observations |
| N Protein | Nucleocapsid Protein |
| NAD ⁺ | Nicotinamide adenine dinucleotide |
| PCS | Post-COVID Syndrome |
| PHQ-8 | Patient Health Questionnaire Depression |
| PSQI | Pittsburgh Sleep Quality Index |
| PSS | Perceived Stress Scale |
| QOD | Questionnaire of Olfactory Disorders |
| RFITT | Risk Factor Intention-to-Treat |
| RFPP | Risk Factor per Protocol |
| S Protein | Spike Protein |
| SAP | Statistical Analysis Plan |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus Type 2 |
| SD | Standard Deviation |
| Self-MOQ | Self-reported Mini Olfactory Questionnaire |



SF-36.....RAND 36-Item Health Survey
T3MS.....Telephone Adaptation of the Modified Mini-Mental State Exam
UPSIT..... University of Pennsylvania Smell Identification Test

Trial Summary

| | |
|-------------------------------|--|
| Principal Investigator | Prof. Dr. Dr. h.c. Stefan Schreiber and Prof. Dr. Matthias Laudes Department of Internal Medicine I University Medical Center Schleswig-Holstein (UKSH), Campus Kiel Arnold-Heller-Str. 3 24105 Kiel Telephone: +49 431 500-22201 Fax: +49 431 500-22204 E-mail: s.schreiber@mucosa.de |
| Sponsor | University Medical Center Schleswig-Holstein (UKSH), Campus Kiel Arnold-Heller-Str. 3 24105 Kiel |
| Title | Improvement of the nutritional status regarding nicotinamide (vitamin B3) and the disease course of COVID-19 |
| Protocol Title | Verbesserung des Ernährungsstatus bezüglich Nicotinamid (Vitamin B3) und Verlauf der COVID-19-Erkrankung |
| Acronym | COVit-2 |
| Background | Nicotinamide, a form of vitamin B3, plays an important role in chronic systemic inflammation and reduced circulating levels impede immune defense against coronaviruses. Results from the pilot study COVit-1 and from the evaluation of the acute phase of the COVit-2 trial suggest that nicotinamide is efficacious against the acute drop in physical performance and other acute symptoms of coronavirus disease 2019 (COVID-19). In the follow-up study after 6 months, the effect of nicotinamide on symptoms of the post-COVID syndrome (PCS) – grouped in a weighted PCS Score or analysed individually – |

| | |
|---------------------------|--|
| | and on levels of antibodies directed against SARS-CoV-2 shall be evaluated. |
| Study Design | Prospective, double-blind, randomized, placebo-controlled clinical trial. |
| Intervention Group | Nicotinamide, 1,000 mg/day p.o. (one each of 500-mg immediate-release and delayed-release nicotinamide tablets), for 4 weeks. |
| Control Group | Placebo, p.o. (2 tablets), for 4 weeks. |
| Randomization | Patients are randomized continuously in a 1:1 ratio according to a randomization scheme in which every second patient is randomized and the following one then receives the opposite allocation. |
| Blinding | Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) |
| Primary Analysis | The primary efficacy variable PCS Score will be compared between the treatment groups using the t-Test or the non-parametric Mann-Whitney U Test. Categorization of PCS Score (none, mild, moderate and severe PCS) between treatment groups will be analyzed using the Fisher-Exact Test. Predictor analysis will be performed to identify predictors for PCS Score at baseline and to define subgroups of patients at risk for PCS. Treatment comparison for this PCS risk subgroup will be performed. |
| Primary Endpoint | Difference between patients treated with nicotinamide and placebo in the PCS Score. |

1. Introduction

The present document aims at detailing the statistical analyses that will be performed for the follow-up after 6 months of the COVIT trial (DRKS00021214, NCT04751604) on the basis of the current study protocol (Version 2.3, dated 13 Dec 2021). This document has been written by the lead statistician and reviewed and agreed by the Principal Investigator of the trial.

In the third year of the SARS-CoV-2 pandemic, COVID-19 still is a large global disease burden resulting in a significant loss in work productivity by both acute and long COVID-19.

Increased catabolism of tryptophan with high levels of kynurenine and other metabolites is a hallmark of acute inflammation upon infection with SARS-CoV-2 (1-3). Degradation of tryptophan results from increased activity of tryptophan-catabolizing enzymes such as indoleamine 2,3-dioxygenase-1 and is associated with disease severity in COVID-19 (1-3), but has also been observed in other infectious diseases including community-acquired bacterial pneumonia (4) and viral infections (5-7). Interestingly, tryptophan absorption also depends on the presence of angiotensin-converting enzyme-2 on the intestinal epithelium, which is also the entry point for SARS-CoV-2 (2, 8). We have previously shown that tryptophan is important to maintain a non-inflammatory homeostasis of the gut microbiome (9) and that supplementation with the tryptophan metabolite nicotinamide conveys strong anti-inflammatory effects that – in animal models – can be transferred with the gut microbiome (9-11). The anti-inflammatory effect through gut-targeted delivery was larger than by systemic supplementation (10).

Infection with SARS-CoV-2 leads to changes of the gut microbiome (12), which are assumed to contribute to COVID-19 outcome, e.g. by licensing of immune responses via microbe-derived metabolites (13, 14). Since impaired tryptophan co-metabolism affects host-microbe homeostasis in COVID-19 patients, topical administration of nicotinamide as a key anti-inflammatory molecule of the pathway should have substantial impact on the COVID-19 microbiome and may serve to improve the course of SARS-CoV-2 infections. Nicotinamide is required for generation of NAD⁺, a coenzyme central to cellular energy metabolism. The availability of NAD⁺ is compromised in infections in general and COVID-19 in particular (15-18). An observational, small open label study has suggested

that COVID-19-related acute kidney injury may be reduced by a 7-day course with 1 g/d nicotinamide (19).

We therefore developed the pharmaceutical pH-dependent matrix tablet formulation CICR-NAM with ingredients approved for use in both food and pharmaceuticals that releases nicotinamide in the lower small intestine and colon to maximize effects on the gut microbiome (NCT05258474).

Following a pilot experiment (COVit-1; DRKS00021214), the prospective, double-blind, randomized, placebo-controlled trial COVit-2 targeted an outpatient population within 7 days after testing PCR-positive for SARS-CoV-2 infection and investigated the effects of one conventional 500-mg nicotinamide tablet plus one 500 mg CICR-NAM tablet administered daily for 4 weeks. Results from COVit-1 and from the evaluation of the acute phase of the COVit-2 trial (until week 6) suggest that nicotinamide is efficacious against the acute drop in physical performance and other acute symptoms of COVID-19.

Reduced organ functions after recovery from acute COVID-19 may persist for weeks to months after the acute phase of the infection in patients following both mild and severe disease courses. There are different terms for this syndrome, such as PCS, post-acute COVID syndrome, post-acute sequelae of SARS-CoV-2 infection or “long COVID”. In the present SAP, the term PCS is used. A large meta-analysis showed that more than half of COVID-19 survivors experienced PCS 6 months after recovery (20). Key symptoms of PCS are fatigue (44% of patients), sleep disorder (33%), dyspnoea (40%), cough (22%) as well as anxiety (34%), depression (32%) and cognitive impairments (15%), and decreased quality of life was reported by 57% of PCS patients (21). Other meta-analyses found that the most frequent persisting symptoms of COVID-19 were fatigue, dyspnoea, exercise intolerance, impaired sense of smell and taste, cognitive impairments, headache, and general pain (22). A recent large trial from Germany reported that “neurological ailments (61.5%), fatigue (57.1%), and sleep disturbance (57.0%) were the most frequent persisting symptoms at 6–12 months after infection” and developed a weighted PCS Score (22).

In the follow-up study of the COVit-2 trial after 6 months, the effect of nicotinamide on symptoms of PCS – as determined by the PCS Score (22) or analysed individually – and on levels of antibodies directed against SARS-CoV-2 shall be evaluated.

2. Study Objectives of the Follow-up Study

2.1. Primary Objective of the Follow-up Study

The primary objective of the follow-up study is to investigate the hypothesis that COVID-19 patients show a reduced intensity of PCS, as determined by the PCS Score (22), in an outpatient setting when supplemented with 1,000 mg of nicotinamide.

2.2. Secondary Objectives of the Follow-up Study

The secondary objectives of the follow-up study are to examine the following parameters: levels of antibodies against the nucleocapsid (N) protein or spike (S) protein of SARS-CoV-2 as well as the occurrence and severity of symptoms characteristic of COVID-19 and/or PCS at the 6-month follow-up, as queried in telephone interviews using the same questions as for the acute phase of COVID-19.

Self-reported Smelling and Tasting Abilities, self-reported Mini-Olfactory Questionnaire (Self-MOQ), Questionnaire of Olfactory Disorders (QOD), Multidimensional Dyspnoea Profile (MDP), Patient Health Questionnaire – Depression (PHQ-8), Generalized Anxiety Disorder 7 (GAD-7), Perceived Stress Scale (PSS), Brief Resilience Scale (BRS), Pittsburgh Sleep Quality Index (PSQI), Multidimensional Fatigue Inventory (MFI), University of Pennsylvania Smell Identification Test (UPSIT) and the T3MS cognitive test will be performed at Month 6 cross-sectionally to investigate different aspects of PCS.

2.3. Exploratory Objectives of the Follow-up Study

Changes in fatigue and quality of life will be exploratively assessed using the questionnaires FACIT-F and SF-36.

3. Trial Methods

3.1. Trial Design

This is a double-blinded, randomized, placebo-controlled parallel group trial.

3.2. Randomization and Blinding

Patients are randomized in one of both treatment groups (nicotinamide or placebo) continuously as they are enrolled. The study is blinded for participants, care providers, investigators and outcome assessors as well as for the statisticians. The staff distributing

the trial supplements is not blinded to ensure proper delivery. Trial supplements are provided in neutral containers. The blinding will not be violated by interim analyses as the allocation of randomization codes to treatment groups is not broken.

3.3. Sample Size

The data available at the beginning of the COVit-2 trial suggested that at most 40% of the already symptomatic COVID-19 patients would be completely symptom-free two weeks after the start of the intervention (primary endpoint defined at that time). This would likely have been the case in the placebo-treated patient population. The hypothesis was that this 40% event rate could be increased to 50% with the use of nicotinamide. Therefore, the following default values were assumed in the primary sample size calculation: α -error = 0.05; power = 0.8; the result is $K = 7.85$ (factor calculated from α -error and power). The following simplified formula was used:

$$n = \frac{K [(R + 1) - p_2 (R^2 + 1)]}{p_2 (1 - R)^2}$$

n = required number of cases

p_1 = event rate in the treatment group (50%)

p_2 = event rate in the control group (40%)

R = relative risk (p_1/p_2) = here: 50% / 40% = 1.25

As a result, 385 patients were needed per group. With an assumed drop-out rate of close to 10%, 420 patients per group were to be included. Based on the results of the futility analysis after $n=400$ patients, the Data Management Board (DMB) of the COVit-2 trial recommended to keep the previously planned sample size of approximately 840 patients and to select the subgroup of patients with at least one risk factor for severe COVID-19 as the primary clinical analysis population. To ensure that the subpopulation recruited after the futility analysis also included an adequate proportion of such patients, the frequencies of risk factors were monitored in a blinded manner and 900 patients were recruited as a safeguard against possibly increased dropout rates. All recruited patients were contacted 6 months after study inclusion.

3.4. Framework

This clinical investigation evaluates the efficacy of a 4-week dietary intervention with a combination of 500 mg immediate-release nicotinamide and 500 mg controlled-ileocolonic release nicotinamide (CICR-NAM) on the recovery from COVID-19 symptoms both in a short-term (up 6 weeks) and a long-term setting (at approximately 6 months). The present SAP describes the analyses pertaining to the long-term setting.

3.5. Investigational Product

The trial uses four investigational products: conventional tablets that start to release nicotinamide immediately after reaching the stomach (immediate-release formulation) as well as novel tablets for CICR-NAM plus a similarly constructed matching placebo. The reasons for applying the innovative tablets for release in the ileum and colon are the gastrointestinal infections with SARS-CoV-2 and recent data showing dysbalances in the intestinal microbiome of COVID-19 patients. CICR-NAM was designed to beneficially influence the intestinal microbiome by topically increasing nicotinamide availability.

3.5.1. Nicotinamide Group

Participants assigned to the nicotinamide group self-administer 2 nicotinamide tablets (1 conventional 500-mg tablet and 1 500-mg tablet with CICR-NAM) for 4 weeks. The overall dose strength of the verum tablets is 1,000 mg nicotinamide, taken once daily with breakfast.

3.5.2. Control Group

Participants randomized to the control group self-administer 2 matching placebo tablets with an appearance corresponding to the respective nicotinamide tablets in the same manner as described above for the nicotinamide group.

3.6. Study Duration and Schedule

The study duration for the individual patient (see schedule below) comprises the 4-week active treatment period, a safety follow-up at 6 weeks and a recall at 6 months with characterization for remaining COVID-19 symptoms (PCS). Recruitment started on 01 February 2021 and the last patient was randomized on 17 January 2022. Week 6 observations were complete in March 2022. After the last 6-month follow-up interview

data had been obtained on 27 July 2022, the last cleaned data from digitalized and double-checked paper questionnaires became available for analysis on 20 October 2022.

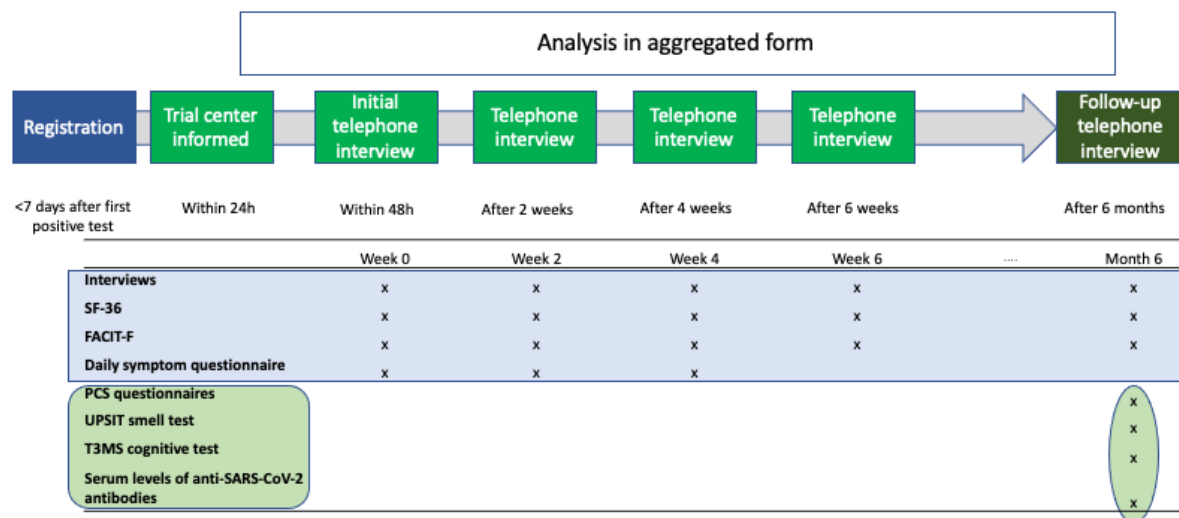


Figure 1: Scheme of visits during the trial.

4. STATISTICAL PRINCIPLES

4.1. Confidence Intervals and P-Values

For all analyses, two-sided 95% confidence intervals (CI) will be reported. Confidence intervals provide an adequately plausible range for the true value related to the measurement of the point estimate. Statements are possible on the direction of the effects, as well as their strength and the presence of a statistically significant result. For (approximately) normally distributed variables, parametric 95% CI will be shown. If this assumption is violated, the corresponding CI will be estimated by bootstrapping methods.

In contrast to confidence intervals, p-values give the difference from a previously specified statistical level α . P-values will be calculated in case of 1) stated hypotheses or 2) in an exploratory fashion to evaluate the difference from a statistical level α . P-values will be shown with three decimal places, whereby p-values less than .05 will be considered as statistically significant. If not otherwise stated, p-values are two-tailed.

4.2. Adherence and Protocol Deviations

The planned analysis will be performed according to the clinical investigation plan, its amendments, and this SAP. If there are contradictions between the clinical investigation plan or its amendments and this SAP, this analysis plan will prevail.

If a protocol deviation or concurrent illness / adverse event occurs, which, according to the clinical judgment of the Investigator, may invalidate the study by pharmacokinetic or pharmacodynamic interference with the trial products, the subject will be withdrawn by the Investigator.

4.3. Analysis Populations

The safety population including all randomized patients has been addressed in the separate SAP for the final analyses of acute COVID-19 (baseline until week 6) dated 27 July 2022. In that SAP, the following three analysis populations were already defined.

The intention-to-treat (ITT) population includes all randomized subjects who have received at least one dose of any study treatment.

According to the recommendations of the DMB after the planned futility analysis, the primary analysis population for efficacy analysis in acute COVID-19 was the **RFITT** population. The RFITT population is defined as all subjects of the ITT population with a least one characteristic or underlying medical condition associated with an increased risk of developing severe illness from COVID-19, selected from the group consisting of

- an age of ≥ 60 years;
- a body mass index of ≥ 30.0 ;
- type 1 diabetes;
- type 2 diabetes;
- cardiovascular diseases;
- high blood pressure;
- stroke;
- asthma;
- chronic obstructive pulmonary disease;
- other chronic lung diseases;

- current or former smokers (the latter being defined as patients who smoked more than 100 cigarettes or other smoking products in total so far, but have not smoked for at least 4 weeks);
- chronic liver diseases;
- chronic kidney diseases;
- cancer;
- organ transplants;
- current immunosuppressive therapy;
- chronic neurological diseases (multiple sclerosis, Parkinson's disease);

The **RFPP** population (see separate SAP for the evaluation of the acute trial phase of COViT-2) is defined as all subjects of the RFITT population, excluding those patients who

- drop out or
- do not comply regarding investigational product intake for at least 80%, *i.e.* intake of investigational product for at least 11 of 14 days between the interventional study intervals (week 0 – week 2 and week 2 – week 4).

The ITT population will be the primary analysis population for efficacy in the 6-month follow-up described here, RFITT and RFPP will be secondary analysis populations.

At the follow-up after 6 months, the primary analysis will also focus on those patients who responded to nicotinamide or placebo treatment in any one of the primary and three key secondary endpoints during acute COVID-19 (until week 6) in order to investigate whether the acute treatment benefit also reduces the occurrence of PCS in these patients (Responder population).

Moreover, in order to identify patients at risk for developing PCS, baseline characteristics and symptoms that are significantly ($P < 0.05$) correlated with the presence of PCS at the 6-month follow-up will be identified in the placebo group. Patients with at least 5 of these predictors will be included in PCS risk subgroups.

In all populations, subgroups of patients with a PCS score (22) of > 0 will be compared between nicotinamide- and placebo-treated patients in addition to the broader analysis of all patients including those with a PCS score of 0. Moreover, a subgroup of patients with a PCS score of ≥ 5 in the RFITT population will be analysed.

5. TRIAL POPULATION

5.1. Eligibility

5.1.1. Inclusion Criteria

- SARS-CoV-2 infection confirmed by laboratory findings; the positive test must not date back more than 7 days.
- Relevant symptoms of a SARS-CoV-2 infection, e.g. in the respiratory or gastrointestinal tract.
- The patient has been able to give written consent via a website before any trial procedure is performed and can comply with the trial-dependent prerequisites and requirements.
- The patient is 18 years or older.

5.1.2. Exclusion Criteria

Exclusion criteria are vaccination against SARS-CoV-2 before inclusion, current participation in another study and pregnancy or breast-feeding.

5.2. Recruitment

A flow diagram showing the progress through the phases of the trial (enrolment, intervention allocation, follow-up, and data analysis) will be produced for both treatment groups.

CONSORT 2010 Flow Diagram

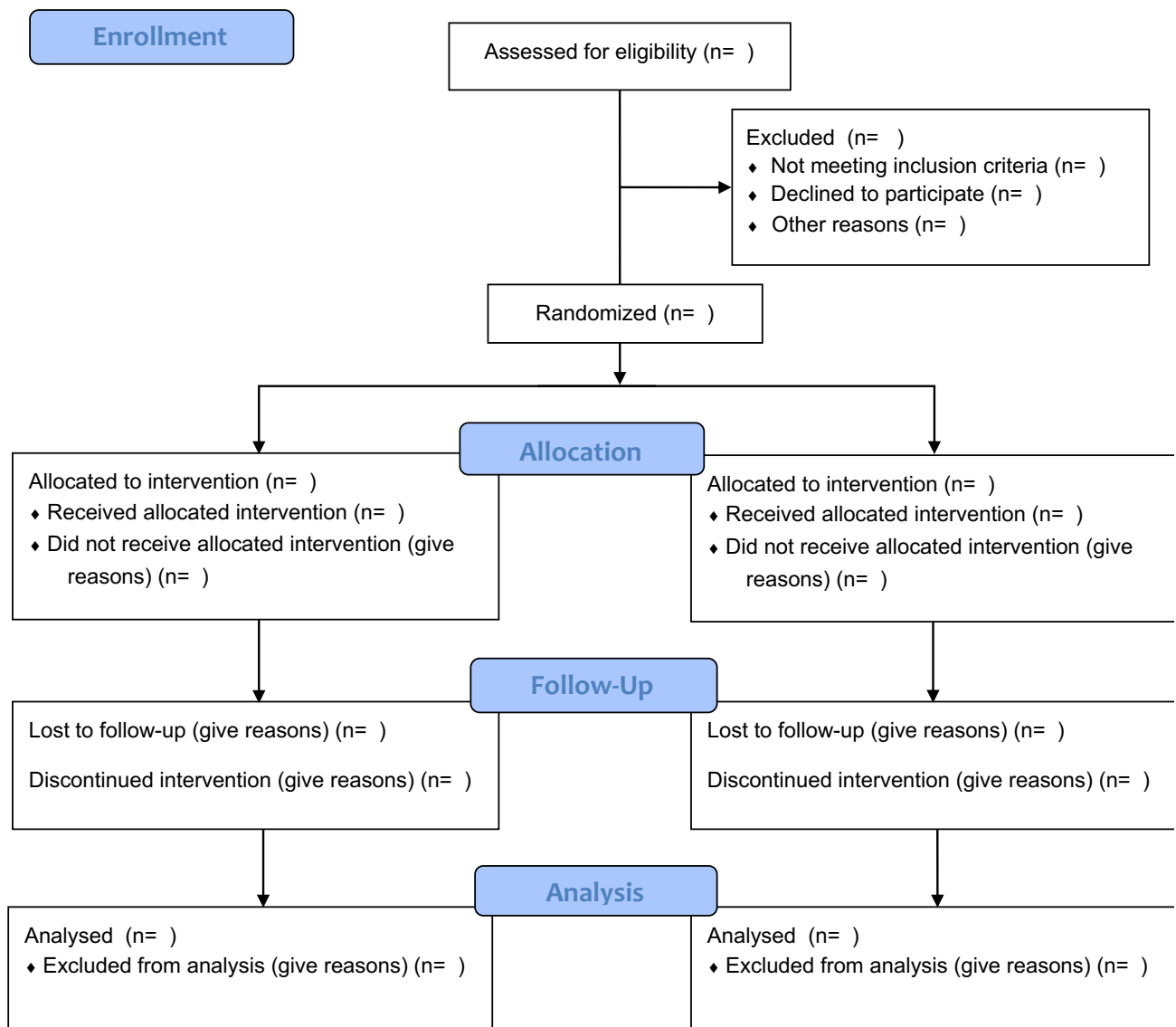


Figure 2 Flow Chart according to CONSORT Requirements.

5.3. Withdrawal/Follow-up

All subjects with evaluable data will be used for each analysis, irrespective of whether or not the subject subsequently dropped out.

The number of drop-outs and withdrawals will be reported as well as the time spent on the study (in days since enrolment) for those participants not completing the study protocol and full follow-up.

6. ANALYSIS

6.1. Outcome Definition

6.1.1. Primary Outcome at the 6-month follow-up

Occurrence and severity of symptoms of PCS measured by the PCS Score (22) between patients treated with placebo and nicotinamide.

6.1.2. Secondary Outcomes at the 6-month follow-up

Anti-SARS-CoV-2 antibodies:

The key secondary endpoint are the serum levels of antibodies directed against the N or S proteins of SARS-CoV-2.

Symptom occurrence and severity:

Symptoms characteristic of COVID-19 and/or PCS, as queried in telephone interviews at the 6-month follow-up using the same questions as for the acute phase of COVID-19.

Frequencies and severity of symptoms that are characteristic for PCS:

overall scale and subscales of

- Self-reported smelling and tasting abilities;
- Self-reported Mini Olfactory Questionnaire (Self-MOQ);
- Questionnaire of Olfactory Disorders (QOD);
- Multidimensional Dyspnoea Profile (MDP);
- Patient Health Questionnaire – Depression (PHQ-8);
- Generalized Anxiety Disorder 7 (GAD-7);
- Perceived Stress Scale (PSS);
- Brief Resilience Scale (BRS);
- Pittsburgh Sleep Quality Index (PSQI);
- Multidimensional Fatigue Inventory (MFI).

University of Pennsylvania Smell Identification Test (UPSIT): overall scale and subscales.

T3MS cognitive test: overall scale and subscales.

6.1.3. Exploratory Outcomes at the 6-month follow-up

Changes in fatigue and quality of life will be exploratively assessed using the following questionnaires:

- Description of the 40-item Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F) questionnaire and its subscales;
- Description of the SF-36 questionnaire (RAND 36-Item Health Survey 1.0) and its subscales.

6.2. Baseline Patient Characteristics

Demographic characteristics of patients available at the follow-up phase will include

- Age;
- Gender;
- BMI (from height and weight);
- Ethnicity;
- Risk factors for severe COVID-19, as described in Section 4.3.

Demographic and baseline characteristics will be summarized descriptively by treatment group for the ITT, RFITT and RFPP analysis set. Continuous variables will be summarized by number of subjects, mean (M), standard deviation (SD), median, minimum, and maximum values. Categorical variables will be summarized by number and percentage of subjects in each category. Comparability of treatment groups will be assessed based on the review of statistical summaries, such as mean/median or percentages in each category, among baseline characteristics and statistical testing (Chi-Square/Fisher Exact test for categorical variables, t-Test or Mann-Whitney-U test for metric variables).

7. ANALYSIS METHODS

7.1. Pre-Processing

7.1.1. PCS Score

The PCS Score will be calculated from patient interview data according to Bahmer *et al.* (22). Long-term symptom complexes underlying the PCS Score definition are shown in Table 1.

Table 1 PCS Score development and weight (see Bahmer et al. (22))

| Symptom complex | Self-reported sub-symptoms at month 6 (present(1)/absent(0)) | Variable from the COVit-2 eCRF | PCS Score weight |
|--------------------------------|--|---|-------------------------|
| Chemosensory deficits | Smelling disturbance, impaired sense of taste | covterma24, covterma25 | 3.5 |
| Fatigue | Fatigue, performance drop | covterma1, covterma2 | 7 |
| Exercise intolerance | Shortness of breath, reduced exercise capacity | covbreath >3, covterma5 | 4 |
| Joint or muscle pain | Muscle pain, joint pain | covterma13, covterm14, covterma15 | 6.5 |
| Ear-Nose-Throat (ENT ailments) | Hoarseness, sore throat, running nose | covterma9, covterma11, covterma12 | 5.5 |
| Coughing, wheezing | Coughing, wheezing | covterma6, covterma7 | 7 |
| Chest pain | Chest pain | covterma16 | 3.5 |
| Gastrointestinal ailments | Stomach pain, diarrhoea, vomiting, nausea | covterma18, covterma19, covterma20, covterma21 | 5 |
| Neurological ailments | Confusion, vertigo, headache, motor deficits, sensory deficits, numbness, tremor, deficits of concentration, cognition or speech | covterma17, covterma26, covterma27, covterma31 | 6.5 |
| Dermatological ailments | Hair loss, rash, itchiness | covterma29, covterma30 | 2 |

| Symptom complex | Self-reported sub-symptoms at month 6 (present(1)/absent(0)) | Variable from the COVit-2 eCRF | PCS Score weight |
|-------------------|--|---|------------------|
| Infection signs | Chills, fever, general sickness/flu-like symptoms | covterma3, covterma4, covterma34, covfeel >3 | 3.5 |
| Sleep disturbance | Insomnia, unrestful sleep | covterma32, covterma33, covsleep >3 | 5 |

For each symptom complex, the presence of at least one of the patient-reported corresponding symptoms will be coded as value 1 (present) or 0 (absent) on the patient level.

The PCS Score will be calculated as weighted sum of the presence or absence of each symptom complex on the patient level using the weight shown in Table 1. The PCS Score ranges from 0 – 59 with higher values indicating more severe PCS.

Patients will be divided according to the PCS Score into ‘none/mild’, ‘moderate’, and ‘severe’ cases using the thresholds for the PCS Score of 10.75 and 26.25, respectively (22).

7.1.2. Level of antibodies directed against the N or S proteins of SARS-CoV-2

Prior to analyses, the time between the positive SARS-CoV-2 PCR test and the date of blood sampling for antibody analysis as well as, if applicable, the time(s) between one or more subsequent vaccinations against SARS-CoV-2 and the sampling date will be calculated in days. Antibody levels are measured using the AProof Duo Test (Adversis Pharma, Leipzig, Germany). In this test, dried blood spot samples are rehydrated and analysed by ELISAs detecting antibodies against the N protein (23) or S protein (24) of SARS-CoV-2. Anti-N antibody levels are quantified as percent of the signal of a positive control. The detection is considered negative if below 20% of the positive control, borderline for 20–30% and positive above 30%. Anti-S antibody levels are quantified as

binding antibody units per milliliter (BAU/mL; according to WHO International Standard for anti-SARS-CoV-2 immunoglobulin [human] [NIBSC code 20/136]). The detection is considered negative if below 22 BAU/mL, borderline for 22–44 BAU/mL and positive above 44 BAU/mL.

7.1.3. Interview Data

Differences between month 6 to baseline (week 0) will be calculated as: month 6 – week 0. For ordinal variables (e.g., symptoms quantified by the complaint scale), the difference is a metric variable. For binary variables [symptom present (yes/no)], the change to baseline will be divided into the categories worsening (symptom not present at week 0 but at month 6), persistence (symptom present at baseline and still present at month 6) or resolution (symptom present at baseline and absent at month 6).

7.1.4. Self-reported Smelling and Tasting Abilities

For the self-reported smelling and tasting abilities, no formal score will be calculated. Changes between the status before, during and after infection will be described by combining items for smelling and tasting. The corresponding data will be exported directly from the database.

| Variable | Scale | Variable name | Min | Max | Direction |
|--|-----------------|------------------|-----|-----|------------------------------------|
| Self-reported smelling before COVID-19 | Scale (ordered) | riech_vor_covid | 0 | 10 | Higher score means better smelling |
| Self-reported smelling during COVID-19 | Scale (ordered) | riech_bei_covid | 0 | 10 | Higher score means better smelling |
| Self-reported smelling after COVID-19 | Scale (ordered) | riech_nach_covid | 0 | 10 | Higher score means better smelling |

| Variable | Scale | Variable name | Min | Max | Direction |
|---|-----------------|---------------------|-----|-----|---|
| Smelling difference after to before COVID-19 | Scale (ordered) | DiffRiechen | -10 | 10 | Negative values indicate worsening, positive values improvement |
| Self-reported nasal breathing before COVID-19 | Scale (ordered) | nasenatm_vor_covid | 0 | 10 | Higher score means better nasal breathing |
| Self-reported nasal breathing during COVID-19 | Scale (ordered) | nasenatm_bei_covid | 0 | 10 | Higher score means better nasal breathing |
| Self-reported nasal breathing since COVID-19 | Scale (ordered) | nasenatm_nach_covid | 0 | 10 | Higher score means better nasal breathing |
| Nasal breathing difference after to before COVID-19 | Scale (ordered) | DiffNasenatm | -10 | 10 | Negative values indicate worsening, positive values improvement |

| Variable | Scale | Variable name | Min | Max | Direction |
|---|-----------------|----------------------|-----|-----|--|
| Self-reported smelling since COVID-19 | Categorical | riech_seit_covid | 0 | 1 | 0: no change, 1: change in smelling ability, -1 unknown |
| Change in smelling | categorical | gerueche | 1 | 3 | 1: less than before, 2: different from before, 3: same as before, -1 unknown |
| Self-reported tasting before COVID-19 | Scale (ordered) | schmecken_vor_covid | 0 | 10 | Higher score means better tasting |
| Self-reported tasting during COVID-19 | Scale (ordered) | schmecken_bei_covid | 0 | 10 | Higher score means better tasting |
| Self-reported tasting after COVID-19 | Scale (ordered) | schmecken_nach_covid | 0 | 10 | Higher score means better tasting |
| Tasting difference after to before COVID-19 | Scale (ordered) | DiffSchmecken | -10 | 10 | Negative values indicate worsening, positive values improvement |

| Variable | Scale | Variable name | Min | Max | Direction |
|---|-----------------|----------------------|-----|------|---|
| Self-reported tasting since COVID-19 | Categorical | schmecken_seit_covid | 0 | 1 | 0: no change, 1 change in tasting ability, -1 unknown |
| Sweet taste | binary | schmecken_suess | 0 | 1 | 0: no change, 1: Change in taste |
| Duration of change in sweet taste (days) | Scale (ordered) | schmecken_suess1 | 0 | 1000 | |
| Salt taste | binary | schmecken_salzig | 0 | 1 | 0: no change, 1: Change in taste |
| Duration of change in salt taste (days) | Scale (ordered) | schmecken_salzig1 | 0 | 1000 | |
| Acid taste | binary | schmecken_sauer | 0 | 1 | 0: no change, 1: Change in taste |
| Duration of change in acid taste (days) | Scale (ordered) | schmecken_sauer1 | 0 | 1000 | |
| Bitter taste | binary | schmecken_bitter | 0 | 1 | 0: no change, 1: Change in taste |
| Duration of change in bitter taste (days) | Scale (ordered) | schmecken_bitter1 | 0 | 1000 | |

| Variable | Scale | Variable name | Min | Max | Direction |
|---|--------------------|-------------------|-----|------|--|
| Pungent taste | binary | schmecken_scharf | 0 | 1 | 0: no change, 1: Change in taste |
| Duration of change in pungent taste (days) | Scale (ordered) | schmecken_scharf1 | 0 | 1000 | |

7.1.5. Self-reported Mini Olfactory Questionnaire (Self-MOQ)

Five items of the Self-MOQ total score are recorded (s_moq1 to s_moq5). The total Self-MOQ Score will be calculated by summing up the number of items answered with yes (25).

| Variable | Scale | Variable name | Min | Max | Direction |
|----------------|---------------|---------------|-----|-----|--|
| Self-MOQ score | Scale (count) | Self_MOQ | 0 | 5 | Lower score means better olfactory function |

7.1.6. Questionnaire of Olfactory Disorders (QOD)

Seven key items of the QOD are answered by the patients. Patients who report disturbance of the sense of smell could agree (3 points), rather agree (2 point), rather disagree (1 point) or disagree (0 points) with each statement. The points of the 7 variables (variable names: qod1, qod13, qod27, qod33, qod37, qod42 and qod49) will be summed up to the total score (26). The initial question qod (“I have a disturbed sense of smell”) with the possible answers *yes*, *no* or *unknown* serves as a filter variable. Only those patients with an impaired sense of smell according to this variable are eligible to fill out the QOD questionnaire.

| Variable | Scale | Variable name | Min | Max | Direction |
|-----------|---------------|---------------|-----|-----|-------------------------------------|
| QOD score | Scale (count) | QOD | 0 | 21 | Higher score means more limitations |

7.1.7. Multidimensional Dyspnoea Profile (MDP)

Elements of the MDP (27, 28) are recorded by the patients to assess overall breathing discomfort (A1), intensity of five sensory qualities (physical breathing effort, air hunger, tightness, mental breathing effort and hyperpnoea) and intensity of emotional responses associated with dyspnoea (i.e. anxious, depressed, angry, frustrated, afraid) (in scales from 0 to 10, with higher values representing more discomfort).

The scores of the domains will be calculated using the arithmetic mean of the corresponding items within each domain. The immediate global unpleasantness item (A1) is used as the leading component of the definition of dyspnoea as “a subjective experience of breathing discomfort” (28).

| Variable | Scale | Variable name | Min | Max | Direction |
|---|---------|----------------------|-----|-----|--|
| A1: Breathing discomfort | ordinal | mdp_a1 | 0 | 10 | Higher values indicate higher discomfort |
| SQ: Physical breathing effort SQ: Air hunger SQ: Tightness SQ: Mental breathing effort SQ: Hyperpnoea | ordinal | mdp_sq_1 to mdp_sq_5 | 0 | 10 | Higher values indicate higher discomfort |
| A2: Depressed A2: Anxious A2: Angry | ordinal | mdp_a2_1 to mdp_a2_5 | 0 | 10 | Higher values indicate higher discomfort |

| Variable | Scale | Variable name | Min | Max | Direction |
|--|--------|---------------|-----|-----|--|
| A2: Frustrated A2: Afraid | | | | | |
| Immediate Perception Domain: Mean (mdp_sq_1 to mdp_sq_5, mdp_a1) | metric | mdp_sq | 0 | 10 | Higher values indicate higher discomfort |
| Emotional Response Domain: Mean (mdp_a2_1 to mdp_a2_5) | metric | mdp_a2 | 0 | 10 | Higher values indicate higher discomfort |

7.1.8. Patient Health Questionnaire – Depression (PHQ-8)

The PHQ-8 total score (29) will be calculated within the database system.

| Variable | Scale | Variable name | Min | Max | Direction |
|-----------------------------|-------------|------------------|-----|-----|--|
| PHQ-8 score | Scale (Sum) | phq8_total_score | 0 | 24 | Higher values indicate higher depression |
| Limitation of activities | Categorical | phq8_1_8 | 0 | 3 | Higher values indicate higher limitation |

7.1.9. Generalized Anxiety Disorder 7 (GAD-7)

The GAD-7 total score (30) will be calculated within the database system.

| Variable | Scale | Variable name | Min | Max | Direction |
|-------------|-------------|------------------|-----|-----|---------------------------------------|
| GAD-7 score | Scale (Sum) | gad7_total_score | 0 | 21 | Higher values indicate higher anxiety |

7.1.10. Perceived Stress Scale (PSS)

The PSS total score (31) will be calculated within the database system.

| Variable | Scale | Variable name | Min | Max | Direction |
|-------------------------|------------|-------------------|-----|-----|--|
| PSS total score | Scale(sum) | pss_gesamt | 10 | 50 | Higher values indicate higher stress level |
| PSS score self-efficacy | Scale(sum) | pss_skala_selbstw | 4 | 20 | Higher values indicate lower self-efficacy |
| PSS score helplessness | Scale(sum) | pss_skala_hilfl | 6 | 30 | Higher values indicate higher helplessness |

7.1.11. Brief Resilience Scale (BRS)

The BRS consists of 6 items (32). Response options are coded on a 5-level Likert scale from 1 (strongly disagree) to 5 (strongly agree) or vice versa, depending on the question. The total score will be calculated before data analysis as arithmetic mean of the 6 items (named brs1 to brs6).

| Variable | Scale | Variable name | Min | Max | Direction |
|-----------|----------------------------|---------------|-----|-----|--|
| BRS score | Scale (arithmetic mean) | BRS | 0 | 5 | Higher values indicate better resilience |

7.1.12. Pittsburgh Sleep Quality Index (PSQI)

The scale and four subscales of PSQI (sleep latency, sleep duration, sleep efficiency and sleep disturbance) will be calculated within the database system (33).

| Variable | Scale | Variable name | Min | Max | Direction |
|--|-------|----------------------|-----|-----|---|
| PSQI score | Scale | psqi_total_score | 0 | 21 | Higher values indicate lower sleep quality |
| PSQI sleep latency | Scale | psqi_schlaflatenz | 0 | 3 | Higher values indicate higher sleep latency (more time) |
| PSQI sleep duration | Scale | psqi_schlafdauer | 0 | 3 | Higher values indicate less sleep |
| PSQI sleep efficiency (duration of sleep divided by duration of bed rest) | Scale | psqi_schlafeffizienz | 0 | 3 | Higher values indicate higher sleep efficacy |

| Variable | Scale | Variable name | Min | Max | Direction |
|---|-------|------------------|-----|-----|---|
| PSQI sleep disturbance (sum of 9 reasons for sleep disturbance) | Scale | psqi_schlafstoer | 0 | 3 | Higher values indicate more sleep disturbance |

7.1.13. Multidimensional Fatigue Inventory (MFI)

The scale and five subscales (general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue) of the MFI will be calculated within the database system (34).

| Variable | Scale | Variable name | Min | Max | Direction |
|------------------------------|-------|------------------------|-----|-----|--|
| MFI general fatigue score | Scale | mfi_general_fatigue | 4 | 20 | Low values indicate lower general fatigue |
| MFI physical fatigue score | Scale | mfi_physical_fatigue | 4 | 20 | Low values indicate lower physical fatigue |
| MFI reduced activity score | Scale | mfi_reduced_activity | 4 | 20 | Low values indicate higher physical activity |
| MFI reduced motivation score | Scale | mfi_reduced_motivation | 4 | 20 | Low values indicate higher motivation |
| MFI mental fatigue score | Scale | mfi_mental_fatigue | 4 | 20 | Low values indicate lower mental fatigue |

| Variable | Scale | Variable name | Min | Max | Direction |
|-----------------|-------|-----------------|-----|-----|---|
| MFI total score | Scale | mfi_total_score | 20 | 100 | Low values indicate lower total fatigue |

7.1.14. University of Pennsylvania Smell Identification Test (UPSIT)

The UPSIT Scale represents the number of correct identified smells with a maximum of 40 and a minimum of 0 (35). The scale will be calculated within the database system.

| Variable | Scale | Variable name | Min | Max | Direction |
|------------------|-------|-----------------|-----|-----|--|
| Smell test score | Scale | Antwort Score.x | 0 | 40 | Higher values indicate higher smelling ability |

7.1.15. T3MS Cognitive Test

The unadjusted scale of the T3MS (Telephone Adaptation of the Modified Mini-Mental State Exam) will be calculated within the database system (36, 37). Adjustments according to years of education may be made during further analyses, as appropriate.

| Variable | Scale | Variable name | Min | Max | Direction |
|------------------------|-------|-----------------|-----|-----|---|
| T3MS score | Scale | Antwort Score.y | 0 | 100 | Higher values indicate higher cognitive ability |
| 3MS age adjusted score | Scale | Corrected Score | 0 | 100 | Higher values indicate higher cognitive ability |

7.1.16. FACIT-F

For the FACIT-F questionnaire, the subscales physical well-being, social/familiar well-being, emotional well-being, functional well-being, fatigue subscale, FACIT-F Trial

Outcome Index (TOI), FACT-G total score and FACIT-F total score will be calculated after double data entry and cleaning within an external Microsoft Excel scoring sheet as described in the manual (38). Items that are left blank (missing data) are not taken into account when calculating the scale scores. Hence, scale scores represent the average for all items in the scale that the respondent answered. Analyses are performed in accordance with the separate SAP for the final analyses of acute COVID-19 (baseline until week 6) dated 27 July 2022.

| Variable | Scale | Min | Max | Direction |
|--------------------------|------------|-----|-----|---|
| Physical well-being | Scale(sum) | 0 | 28 | Higher values indicate greater well-being |
| Social/family well-being | Scale(sum) | 0 | 28 | Higher values indicate greater well-being |
| Emotional well-being | Scale(sum) | 0 | 24 | Higher values indicate greater well-being |
| Functional well-being | Scale(sum) | 0 | 28 | Higher values indicate greater well-being |
| Fatigue subscale | Scale(sum) | 0 | 52 | Higher values indicate greater well-being |
| FACIT-F TOI | Scale(sum) | 0 | 108 | Higher values indicate greater well-being |
| FACT-G total score | Scale(sum) | 0 | 108 | Higher values indicate greater well-being |
| FACIT-F total score | Scale(sum) | 0 | 160 | Higher values indicate greater well-being |

7.1.17. SF-36

The scoring of the RAND 36-Item Health Survey 1.0 (https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form/scoring.html) (39) will be performed after double data entry and cleaning using an external scoring scheme in Microsoft Excel.

Scale and subscales will be calculated and sent for data analyses. Items that are left blank (missing data) are not taken into account when calculating the scale scores. Hence, scale scores represent the average for all items in the scale that the respondent answered. Analyses are performed in accordance with the separate SAP for the final analyses of acute COVID-19 (baseline until week 6) dated 27 July 2022.

| Variable | Scale | Min | Max | Direction |
|--------------------------------|-------|-----|-----|---|
| Physical functioning | Scale | 0 | 100 | Higher values indicate greater well-being |
| Role functioning/ physical | Scale | 0 | 100 | Higher values indicate greater well-being |
| Role functioning/ emotional | Scale | 0 | 100 | Higher values indicate greater well-being |
| Energy / fatigue | Scale | 0 | 100 | Higher values indicate greater well-being |
| Emotional well-being | Scale | 0 | 100 | Higher values indicate greater well-being |
| Social functioning | Scale | 0 | 100 | Higher values indicate greater well-being |
| Pain | Scale | 0 | 100 | Higher values indicate greater well-being |
| General health | Scale | 0 | 100 | Higher values indicate greater well-being |
| Health change | Scale | 0 | 100 | Higher values indicate greater well-being |

7.2. Main Analysis

The standard summary statistics for continuous baseline and outcome variables will be: N, mean (M), standard deviation (SD), 95% CI, median, minimum and maximum. The standard summary statistics for categorical baseline and outcome variables will be absolute and relative frequencies (expressed as percentage).

Prior to analysis, all metric variables will be checked with respect of the normality assumption to draw reliable interpretations and conclusions of the research. Skewness and kurtosis are used to assess the distribution form. If the absolute values are less than 1, the deviation from the normal distribution is considered safe (40).

Analyses of primary and secondary endpoints will be conducted on the ITT population (primary analysis population, see section 4.3) as well as for the RFITT and RFPP populations. An overview of all analyses is provided in Appendix A.

Analyses of the PCS Score

The primary efficacy variable PCS Score will be compared between the treatment groups using the t-Test or the non-parametric Mann-Whitney U Test. Categorization of the PCS Score (none, mild, moderate and severe PCS) between treatment groups will be analyzed using the Fisher-Exact Test or the Chi-Square Test. All recorded symptoms at month 6 will be listed with absolute and relative frequencies and compared between the two treatment groups using the Fisher-Exact Test or the Chi-Square Test. In case that more than 20% of expected cell counts within the contingency tables are less than 5, the Fisher-Exact Test will be used instead of the Chi-Square Test. Responders of primary and key secondary endpoints within the first 6 weeks (acute COVID-19) will be considered as a subgroup (see section 4.3). The PCS formula will be applied to visits of the acute phase (week 0 to week 6) as a measure of overall COVID-19 symptom severity and will be analyzed descriptively using histograms. The PCS Score over time will be analyzed using a mixed model repeated measures (MMRM) approach with the baseline value as a covariate, fixed effect terms in the model group, week, and group-by-week interaction, and an unstructured covariance matrix. Using this model, the difference in change from baseline between the groups will be estimated based on least square mean difference with corresponding 95% CI and p-value. In the event that the unstructured covariance

matrix is non-estimable (due to lack of convergence) when fitting the MMRM to the observed study data, an autoregression of lag 1 covariance matrix will be used instead. For the comparison of selected visits, contrasts will be used. Individual courses of the PCS Score from baseline to month 6 will be drawn for patients with increased PCS at month 6. Tables with mean and SD will be shown and the t-Test or the non-parametric Mann-Whitney U Test will be applied to evaluate differences between treatment groups. The PCS Score in relation to time of recruitment will be analyzed exploratively to evaluate if severity of PCS is related to time of COVID-19 infection. Analyses will be performed in an exploratory fashion using scatterplots (time of recruitment vs. PCS score) and interpolation using smoothing procedures (locally-weighted scatterplot smoothing (LOESS)). Frequency and severity of PCS in patients with particularly mild progression (symptom-free after 2 weeks) compared to other patients will be performed using the t-Test or the non-parametric Mann-Whitney U Test.

Predictor analyses

Relevant predictors for PCS at month 6 will be obtained by screening baseline parameters (demographics, medical history, concomitant medication, risk factors for severe COVID-19 and symptoms present at baseline) as well as changes in the early stage of the disease (resolution/new onset/constant presence/constant absence of symptoms at week 2 compared to baseline). Screening will be applied within the placebo group comparing patients with a PCS score of ≥ 5 at month 6 with patients having a PCS score of < 5 . The t-test or the non-parametric Mann-Whitney-U test will be performed. Relevant predictors will be identified as parameters with p-values of $< .05$. Subgroups of patients at risk for developing an increased PCS Score will be defined using these relevant predictors. All patients belonging to PCR risk subgroups will be compared with regard to treatment group (nicotinamide or placebo) applying the t-test or the non-parametric Mann-Whitney-U test.

Comparison of symptoms to baseline

Change from baseline values will be analyzed for all symptoms (categorical and ordinal) at month 6. Comparison to baseline situation (symptom resolved at month 6, constant symptom present, symptom absent, symptom newly reported at month 6) will be

calculated and compared between treatment groups using the Fisher-Exact test or the Chi-Square test. For evaluation of symptoms measured on a scale of complaints, change to baseline between treatment groups will be analyzed using Mann-Whitney-U Test or t-test for independent samples.

Analyses of antibody levels

Analyses of antibodies directed against the N or S protein of SARS-CoV-2 will be performed using ANOVA. The level of antibodies against the N or S protein will be the dependent variables, group and number of vaccinations (none, one, multiple) will be independent factors. Post-hoc tests for vaccination will be performed using the Holm procedure to adjust for multiple testing. Correlation analysis showing the correlation coefficient, 95% CI and p-values will be applied to investigate the correlation of time difference between the positive test for SARS-CoV-2 (and/or) study inclusion (date of baseline visit at week 0) and the date of blood sampling for the antibody test. The time between sampling and baseline interview will be compared between the two treatment groups using the t-Test or the non-parametric Mann-Whitney U Test. Responders of primary and key secondary endpoints within the first 6 weeks (acute COVID-19) will be considered as a subgroup (see section 4.3). Correlation of the levels of antibodies against N or S protein and the PCS Score will be performed using the Pearson correlation coefficient or the Spearman correlation coefficient (in case of a violation of the normality assumption). Threshold values for negative, borderline or positive antibody detection as described in section 7.1.2 will be applied and treatment comparisons for these categorizations will be performed for all patients (ITT, RFITT, RFPP, Responder), as well as the subgroups of vaccinated and unvaccinated patients.

Analyses of questionnaires and tests

For the patient questionnaires (Self-MOQ, QOD, MDP, PHQ-8, GAD-7, PSS, BRS, PSQI, MFI, UPSIT, T3MS, FACIT-F and SF-36), the t-test or the Mann-Whitney-U test will be applied to test differences in total score or subscores between treatment groups.

Patients who reported impaired sense of smell or taste within the interviews will be compared with patients without impaired sense of smell or taste with respect to their

results in Self-MOQ, QOD, UPSIT and self-reported smelling/tasting using the t-test or the Mann-Whitney-U test.

All statistical tests will be 2-tailed with a type I error of .05 unless otherwise stated.

7.3. Subgroups

Subgroup analyses will be performed on demand in order to further define responders. Possible subgroups might be defined in accordance with the futility analysis and the analysis of acute COVID-19:

1) Risk groups as subgroups of the ITT, RFITT and RFPP populations:

- age of ≥ 60 years;
- body mass index of ≥ 30.0 or type 2 diabetes;
- cardiovascular diseases;
- high blood pressure;
- stroke;
- asthma, chronic obstructive pulmonary disease or other chronic lung diseases;
- current or former smokers (the latter being defined as patients who smoked more than 100 cigarettes or other smoking products in total so far, but have not smoked for at least 4 weeks).

2) Booster vaccination (yes/no, once/twice).

3) Responder in any one of the primary and key secondary endpoints during acute COVID-19 (until week 6; for details see the separate SAP for the final analyses of acute COVID-19 dated 27 July 2022).

4) Patients at risk for the development of PCS according to predictors identified in the placebo arm of the trial.

Subgroup analyses will be performed using the methods described in section 7.2.

7.4. Final Analyses and Reporting

The final analyses described in this SAP will be performed after the database has been cleaned and locked (database hard lock).

7.5. Missing Data

All efforts will be made to achieve complete capture of all data from all patients. Data of the primary outcome will be captured by interviews and the interviewers are instructed to contact patients multiple times until they receive feedback from them. Within the dataset, symptoms are reported only in case they are present and could be observed. So, all patients with no reported symptoms and thus missing values are treated as patients with no symptoms. Statistical methods that do not employ imputation like mixed model repeated measures (MMRM) or Chi-Square Test will be used for analyses. The use of the MMRM model assumes implicitly that data are missing at random.

7.6. Multiple Testing Procedure

To counteract the problem of multiple testing, the Bonferroni-Holm method will be applied where appropriate.

7.7. Statistical Software

R version 3.6.3 using RStudio Version 1.1.463 or a more recent version at the time of the analysis will be used for all statistical analyses.

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Appendix A: Summary of Analyses

| Variable | Outcome | Population ^a | Statistical Method | Type |
|---|-------------|--|--------------------------------------|--|
| Analyses of the PCS Score | | | | |
| Differences between treatment groups in the individual PCS Score at month 6 | Metric | ITT, RFITT, RFPP, Responder | t-Test, Mann-Whitney-U | Cross sectional |
| PCS Score during the first 6 weeks of acute COVID-19 incl. comparison of treatment groups | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Time course of PCS Score during the trial incl. comparison of treatment groups | Metric | ITT, RFITT, RFPP, Responder | MMRM | Longitudinal |
| Individual course of PCS for patients with PCS Score >10 at month 6 | Metric | ITT | Scatterplot | Longitudinal |
| Comparison of PCS Score between treatment groups for patients with PCS ≥ 5 | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| PCS Score in relation to time of recruitment (date baseline interview) | Metric | ITT | Scatterplot with LOESS interpolation | Cross sectional |
| PCS Score of patients with a mild disease course (symptom-free after 2 weeks) vs. others | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Predictor analyses | | | | |
| Predictor analysis (2 Stages: a) Screening and extraction of relevant predictors for PCS, b) treatment comparison in the PCS risk subgroups) | Metric | ITT (a) only Placebo), RFITT, PCS Risk subgroups | t-Test, Mann-Whitney-U | Longitudinal using baseline parameters |
| Comparison of patients who reported impaired sense of smell or taste within the interviews with patients without impaired sense of smell or taste with respect to their results in QOD, MOQ, UPSIT and self-reported smelling/tasting | Metric | ITT | t-Test, Mann-Whitney-U | Cross sectional |
| Comparison of symptoms to baseline | | | | |
| Differences in symptoms at month 6 compared to baseline | Categorical | ITT, RFITT, RFPP | Fisher-Exact Test or Chi-Square Test | Cross sectional |
| Differences in symptoms at month 6 compared to baseline (scale of complaints) | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |

| Analyses of antibody level | | | | |
|--|-------------|------------------|---|-----------------|
| Level of antibodies against the N or S proteins of SARS-CoV-2 | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Correlation of PCS Score and antibody levels | Metric | ITT | Pearson or Spearman correlation, Scatterplot, LOESS | Cross sectional |
| Treatment comparison of anti-N and anti-S antibody levels in patients with and without vaccination | Metric | ITT | t-Test, Mann-Whitney-U | Cross sectional |
| Time difference from positive SARS-CoV-2 test or first vaccination to time of sampling of antibodies between treatment groups | Metric | ITT | t-Test, Mann-Whitney-U | Cross sectional |
| Level of antibodies against the N or S proteins of SARS-CoV-2 between groups and vaccination level (none, one, multiple) | Metric | ITT, RFITT, RFPP | ANOVA | Cross sectional |
| Level of antibodies against the N or S proteins of SARS-CoV-2 between groups and vaccination level (none, one, multiple) applying threshold values | Categorical | ITT, RFITT, RFPP | Fisher-Exact Test or Chi-Square Test | Cross sectional |
| Correlation of time from positive SARS-CoV-2 test (or inclusion date) to date of laboratory testing of the level of antibodies against the N or S proteins of SARS-CoV-2 | Metric | ITT, RFITT, RFPP | Correlation | Cross sectional |
| Analyses of questionnaires and tests | | | | |
| Differences between treatment groups in the scale of the Self-MOQ | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale of the QOD | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale and subscales of the MDP | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale of the PHQ-8 | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale of the GAD-7 | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale of the PSS | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |

| | | | | |
|---|--------|------------------|------------------------|-----------------|
| Differences between treatment groups in the scale of the BRS | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale and subscales of the PSQI | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale and subscales of the MFI | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale and subscales of the FACIT-F | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale and subscales of the SF-36 | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale of the UPSIT | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the scale of the T3MS cognitive test | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |
| Differences between treatment groups in the combination of items for smelling and tasting of self-reported smelling and tasting abilities | Metric | ITT, RFITT, RFPP | t-Test, Mann-Whitney-U | Cross sectional |

Annotation: a) Primary analysis population: ITT