Supplementary methods

**Full inclusion criteria**

- 18 years of age or older
- Community acquired pneumonia (defined as a new radiographic infiltrate on chest x-ray or CT scan in a patient presenting with respiratory symptoms both of which are clinically evident less than 48 hours after hospitalization).
- Tested for suspected SARS-CoV-2 infection via RT-PCR or another approved laboratory method*
- Increased risk of mortality on admission (defined by CURB65 score greater than or equal to 1 or the presence of bilateral radiographic infiltrates)
- Treatment can be commenced within 96 hours of hospital admission
- Requires hospitalisation but NOT requiring mechanical ventilation at randomisation
- Participant (or legally authorized representative) provides written informed consent
- Able to take oral medication at randomisation
- Participant (or legally authorised representative) understands and agrees to comply with planned trial procedures.

**Full exclusion criteria**

- Alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) greater than 5 times the upper limit of normal, result within 72 hours of randomisation (the result closest to randomisation should be used if several results are available).
- Stage 4 severe chronic kidney disease or requiring dialysis (i.e. eGFR less than 30), result within 72 hours of randomisation (the result closest to randomisation should be used if several results are available)
- Pregnant or breast feeding.
- Anticipated transfer to another hospital which is not a trial site within 24 hours.
- Hospital-acquired pneumonia (defined as onset of respiratory illness more than 48 hours after admission to hospital)
- Allergy to SFX-01
- Patients in whom active treatment is not considered appropriate.
- Use of any investigational drug within five times of the elimination half-life after the last trial dose or within 30 days, whichever is longer.

Women of child-bearing potential (WOCBP) must be willing to have pregnancy testing prior to trial entry and agree to use approved contraception throughout the trial (see section 8.11).

**Supplementary- dose selection**

Healthy human volunteer trials on SFX-01 established the maximum tolerated dose of SFX-01 of 700mg (containing 107mg sulforaphane) qd. Single doses of 300mg SFX-01 in healthy volunteers was well tolerated in most patients and so previous trials in subarachnoid haemorrhage (NCT02614742) and breast cancer (Eudract 2015-004851-28) used a dose of 300mg BID.

We selected a 300mg once daily dose after review of prior clinical trials of sulforaphane, which have recently been reviewed (Yagishita et al. Molecules. 2019 Oct 6;24(19)). The selected dose is equivalent to the third highest dose that has been used in any clinical trial. This dose is 2.5-fold higher than a dose for which there is clear evidence of target engagement, i.e. activation of Nrf2-target genes and down-regulation of inflammatory markers in PBMCs (Liu et al. Sci Rep. 2020 Apr 2;10(1):5822), and 10-fold higher than the 'internal' dose (based on the 24-h urinary excretion of sulforaphane and its metabolites) that has demonstrated efficacy, i.e. statistically significant increase in detoxication of benzene, an airborne pollutant (Chen et al. Am J Clin Nutr. 2019 Sep 1;110(3):675-684) in human subjects living in areas with unavoidable environmental pollution. We selected a dosing regimen of once a day, because the Nrf2-transcriptional targets, which are the actual protectors, are proteins with long half-lives (several days). On this basis, 300mg SFX-01 (containing 46mg sulforaphane) once daily was used for this trial.

**Supplementary statistical methods**

Based on the WHO master protocol for COVID19 trials (March 2020), outcome data for 272 participants were required to detect an odds ratio of 2.0 on the primary outcome of clinical status at day 15 using 85% power at 5% significance. The target sample size was therefore 300 patients to account for potential drop-outs.

Primary efficacy analyses were based on the ITT population. Safety analyses were based on a modified ITT population consisting of all participants who were randomised and received at least one dose of randomised therapy. A per-protocol analysis was performed including all participants who completed randomly assigned therapy over 14 days. The primary study endpoint based on the WHO 7-point ordinal scale was evaluated using mixed-effects ordinal logistic regression, assuming proportional odds, adjusted for the stratifying factor of CURB65 score as random effect. Results are expressed as odds ratios (OR) where an OR>1 indicates a beneficial effect of treatment with SFX-01 on clinical status. Secondary outcomes of time to event were evaluated using Cox proportional hazards regression and adjusting for CURB65 score. Number of days free from oxygen, new oxygen use, days free from ventilation, new ventilation use and also adverse events between the SFX-01 and placebo groups were analysed using negative binomial regression.

The World Health Organisation (WHO) 7-point ordinal scale was used to assess clinical status at each time point. The worst score for each day was recorded. The National Early Warning Score (NEWS) was measured throughout the participants' hospital stay.

Pre-specified sub-group analyses were performed for the primary outcome based on age, sex, SARS-CoV-2 positivity, detection of pathogens, and subgroups based on WHO scale at baseline.

Serum biomarker data were analysed using mixed-model repeated measures (MMRM) approach where the model includes a fixed effect for treatment group and nominal time (days 1, 8 and 15 after randomisation) and treatment by time interaction with patient included in the model as a random intercept and an unstructured covariance structure assumed. For mRNAseq data, using Wald test and Benjanini-Hochberg procedure, a false discovery rate adjusted p-value of <0.05 with a $Log_2$ fold-change of >1 or <-1 between treatment groups was considered significant.

**Olink Target48 panel serum cytokine analysis**

At day 1 (baseline), 8, and 15 (end-of-trial), venous blood was drawn into an SST™II Advance vacutainer on days 1 s, 8 and 15 and allowed to coagulate at room temperature (RT) for minimum 30 minutes. Serum was obtained by centrifugation at 1000 *g* for 15 min, RT, aliquoted and stored at -80°C until use.

Stored serum samples were thawed in batches of 40-80, centrifuged at 10,000*g* for 5 minutes to remove debris, then 20µl was aliquoted into 96-well V-bottom PCR plates and refrozen at -80°C. Samples were processed for analysis with the qPCR-based Olink Target 48 cytokine panel (Olink, Upsala, Sweden) according to the manufacturer's instructions at ARCADIA, Central Diagnostic Laboratories, University Medical Centre Utrecht (ISO9001) to quantify 45 serum proteins. All study samples from the same participant were run within the same assay plate. For data analysis, concentration values below the lower limit of detection were included. Concentrations above the upper limit of quantification were assigned a value of the maximum detected concentration within the cohort, plus one standard deviation.

**Peripheral blood leukocyte mRNA sequencing**

Venous blood was drawn into PAXgene® Blood RNA Tubes (Qiagen, UK) and tubes were incubated at RT for a minimum of 2h and maximum 18h to allow cell lysis and RNA stabilization, then subsequently stored at -80°C until use. Further processing including RNA extraction, library preparation, mRNA sequencing and differential expression analyses was performed by Azenta US, Inc (South Plainfield, NJ, USA) as follows: total RNA was extracted from blood using Qiagen PAXgene Blood RNA Kit following manufacturer's instructions (Qiagen, Hilden, Germany). Library Preparation with polyA selection, globin depletion and Illumina Sequencing Extracted RNA samples were quantified using Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and RNA integrity was checked using Agilent TapeStation 4200 (Agilent Technologies, Palo Alto, CA, USA). RNA sequencing libraries were prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina following manufacturer's instructions (NEB, Ipswich, MA, USA). Briefly, mRNAs were first enriched with Oligo(dT) beads and globin mRNA was depleted using QIAGEN FastSelect Globin Kit (Qiagen, Hilden, Germany). Enriched mRNAs were fragmented for 15 minutes at 94 °C. First strand and second strand cDNAs were subsequently synthesized. The

second strand of cDNA was marked by incorporating dUTP during the synthesis. cDNA fragments were end repaired and adenylated at 3'ends, and universal adapters were ligated to cDNA fragments, followed by index addition and library enrichment by limited-cycle PCR. The sequencing libraries were validated on the Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA), and quantified by using Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) as well as by quantitative PCR (KAPA Biosystems, Wilmington, MA, USA). The sequencing libraries were multiplexed and loaded on the flowcell on the Illumina NovaSeq 6000 instrument according to manufacturer's instructions. The samples were sequenced using a 2x150 Pair-End (PE) configuration v1.5. Image analysis and base calling were conducted by the NovaSeq Control Software v1.7 on the NovaSeq instrument. Raw sequence data (.bcl files) generated from Illumina NovaSeq was converted into fastq files and de-multiplexed using Illumina bcl2fastq program version 2.20. One mismatch was allowed for index sequence identification.

Gene set enrichment analyses and pathway clustering were done in Rstudio using the clusterProfiler (https://www.liebertpub.com/doi/10.1089/omi.2011.0118) package. Log2FC values and -log10 p-values from the differential expression analyses were used to generate a ranked list of genes by signed p-value, all genes detected were included for analysis. NES >1 and adjusted p-value <0.5 were utilised for pathway statistical significance. STRING v12.0 search tool was used to construct the protein-protein interaction network.

**Exploratory sub-study statistical analyses**

*Serum cytokines*

For data analysis from Olink Target48 cytokine panel, data were analysed using mixed - model repeated measures (MMRM) approach with SPSS software (version 27, IBM). The model included a fixed effect for treatment group and nominal time (1, 8 and 15 days), and treatment–by-time interaction. For serum analyses, the intention-to-treat population was utilized, but patients with recorded administration of tocilizumab were excluded due to significant effects on cytokine levels.

*Trial mRNA sequencing data*

After investigating the quality of the raw data, sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v.0.36. The trimmed reads were mapped to the Homo sapiens reference genome available on ENSEMBL using the STAR aligner v.2.5.2b. The STAR aligner is a splice aligner that detects splice junctions and incorporates them to help align the entire read sequences. BAM files were generated as a result of this step. Unique gene hit counts were calculated by using feature Counts from the Subread package v.1.5.2. Only unique reads that fell within exon regions were counted. After extraction of gene hit counts, the gene hit counts table was used for downstream differential expression analysis. Using DESeq2, a comparison of gene expression between the groups of samples was performed. The Wald test was used to generate p-values and Log2 fold changes. Genes with adjusted p-values <0.05 and absolute log2 fold changes >1 were called as differentially expressed genes for each comparison.

As the objective of this gene expression analysis was to investigate the pharmacological effect of SFX-01 on key NRF2 targets, participants who withdrew from study treatment were excluded.

*Re-analysis of L-SFN-treated PBMC mRNAseq data*

For re-analysis of a published mRNAseq data set from PBMC isolated from 4 human donors and treated with 15 μM L-sulforaphane (L-SFN) for 24h, or receiving no treatment (control), FASTQ files were downloaded from the European Nucleotide Archive (Project: PRJNA672987). *STAR* version 2.7.10a was used to map reads to reference genome GRCh38. *FastQC* v0.11.9 and *FastQ* Screen v0.15.1 were used for quality control. Differential expression was done using *edgeR* version 3.38.4. A false discovery rate(FDR) <0.05 (equivalent to Benjamini-Hochberg adjusted p-value of 0.05) with absolute $\log_2$ fold-change >1 between treatment groups was considered significant. Data using a more stringent FDR <0.01 was also detailed where appropriate. Gene set enrichment was done using *fgsea* version 1.22.0 and GO, Reactome, KEGG and BioPlanet databases. The code used to perform these analyses is available at https://github.com/bartongroup/MG_sulfRNAseq.

Graphs for exploratory analyses were generated with GraphPad Prism (version 9.4.0).

Supplementary results:

**Table s1. Numbers and percentage of participants who received additional treatments.**

| Treatment | Placebo N=68 | SFX-01 N=65 | Total N=133 |
|---|---|---|---|
| Antibiotic | 23 (33.8) | 16 (24.6) | 39(29.3) |
| Corticosteroid | 47 (69.1) | 46 (70.8) | 93(69.9) |
| Others | 46 (67.6) | 42 (64.6) | 88(66.2) |
| Remdesivir | 0 | 1 (1.5) | 1(0.8) |
| Tocilizumab | 8 (11.8) | 12 (18.5) | 20(15.0) |

*Numbers in cells are n(%)

**Subgroup analysis of primary outcome**

Subgroup analysis of the primary endpoint was performed for the following pre-specified groups:

Age (years): (a) <65 years and (b) ≥ 65
Sex at birth: (a) Male and (b) Female
Baseline 7-point ordinal scale: (a) 3, (b) 4 and (c) 5
Symptom duration (days): (a) <10 and (b) ≥10
Other pathogen identified vs no bacterial pathogen identified
COVID test: (a) positive and (b) negative

Age (years): (a) <65 years and (b) ≥ 65

Table s2 shows the subgroup analysis for age. There was no evidence that the treatment effect was moderated by age group: interaction effect 0.84 (0.14, 5.11); p-value = 0.801.

**Table s2: Subgroup analysis for age group**

| | Placebo N=67 | SFX-01 N=65 | Age group | Effect size (99% CI) | p value |
|---|---|---|---|---|---|
| Less than 65 years | | | | | |
| Not hospitalised, no limitations on activities | 3/27(11.1) | 4/42(9.5) | Less than 65 years | 0.66(0.19,2.26) | 0.387 |
| Not hospitalised, limitations on activities | 21/27(77.8) | 29/42(69.0) | 65 years or more | 0.55(0.15,2.11) | 0.257 |
| Hospitalised, not requiring supplemental oxygen | 1/27(3.7) | 1/42(2.4) | Interaction | 0.84(0.14,5.11) | 0.801 |

| | | |
|---|---|---|
| Hospitalised, requiring supplemental oxygen | 1/27(3.7) | 0 |
| Hospitalised, on non-invasive ventilation or high flow oxygen devices | 0 | 1/42(2.4) |
| Death | 1/27(3.7) | 7/42(16.7) |
| 65 years and more | | |
| Not hospitalised, no limitations on activities | 8/41(19.5) | 2/23(8.7) |
| Not hospitalised, limitations on activities | 17/41(41.5) | 11/23(47.8) |
| Hospitalised, not requiring supplemental oxygen | 5/41(12.2) | 1/23(4.3) |
| Hospitalised, requiring supplemental oxygen | 0 | 1/23(4.3) |
| Death | 10/41(24.4) | 8/23(34.8) |
| Missing | 1/41(2.4) | |

*Adjusted for minimization variable; CURB score

Sex at birth: (a) Male and (b) Female

Table s3 shows the subgroup analysis for gender. There was no evidence that the treatment effect was moderated by gender: interaction effect 1.35 (0.23, 7.97); p-value = 0.661.

**Table s3: Subgroup analysis for gender**

| | Placebo N=68 | SFX-01 N=65 | Gender | Effect size (99% CI) | p value |
|---|---|---|---|---|---|
| Male | | | | | |
| Not hospitalised, no limitations on activities | 9/42(21.4) | 5/36(13.9) | Male** | 0.64(0.19,2.15) | 0.345 |
| Not hospitalised, limitations on activities | 22/42(52.4) | 21/36(58.3) | Female** | 0.87(0.24,3.19) | 0.780 |
| Hospitalised, not | 3/42(7.1) | 0 | Interaction | 1.35(0.23,7.97) | 0.661 |

| | | |
|---|---|---|
| requiring supplemental oxygen | | |
| Hospitalised, requiring supplemental oxygen | 1/42(2.4) | 1/36(2.8) |
| Hospitalised, on non-invasive ventilation or high flow oxygen devices | 0 | 1/36(2.8) |
| Death | 6/42(14.3) | 8/36(22.2) |
| Missing | 1/42(2.4) | 0 |
| | | |
| Female | | |
| Not hospitalised, no limitations on activities | 2/26(7.7) | 1/29(3.4) |
| Not hospitalised, limitations on activities | 16/26(61.5) | 19/29(65.5) |
| Hospitalised, not requiring supplemental oxygen | 3/26(11.5) | 2/29(6.9) |
| Death | 5/26(19.2) | 7/29(24.1) |

*Adjusted for minimization variable; CURB score

**One male patient was misclassified as female in the SFX01 group and the error identified after database lock and completion of analysis. The percentages have been corrected but results of the pre-specified ordinal logistic regression subgroup analyses have not been corrected.

Baseline 7-point ordinal scale: (a) 3, (b) 4 and (c) 5

Table s4 shows the subgroup analysis for baseline 7-point ordinal scale. There was no evidence that the treatment effect was moderated by baseline ordinal scale: interaction effect 1: 0.22 (0.03, 1.62); p-value = 0.051 and interaction 2: 0.87 (0.05, 16.40); p-value=0.901.

**Table s4: Subgroup analysis for baseline 7-point ordinal scale**

| | Placebo N=68 | SFX-01 N=65 | Baseline CSTAT | Effect size (99 CI) | p value |
|---|---|---|---|---|---|
| Baseline CSTAT = 3 | | | | | |
| Not hospitalised, no limitations on activities | 4/28(14.3) | 5/24(20.8) | CSTAT = 3 | 1.66(0.39,6.99) | 0.364 |
| Not hospitalised, limitations on activities | 19/28(67.9) | 17/24(70.8) | CSTAT = 4 | 0.37(0.10,1.40) | 0.055 |
| Hospitalised, not requiring supplemental oxygen | 3/28(10.7) | 1/24(4.2) | Interaction 1 | 0.22(0.03,1.62) | 0.051 |
| Death | 2/28(7.1) | 1/24(4.2) | CSTAT = 5 | 0.69(0.05,9.01) | 0.714 |
| | | | Interaction 2 | 0.87(0.05,16.40) | 0.901 |
| Baseline CSTAT= 4 | | | | | |
| Not hospitalised, no limitations on activities | 6/33(18.2) | 1/30(3.3) | | | |
| Not hospitalised, limitations on activities | 17/33(51.5) | 17/30(56.7) | | | |
| Hospitalised, not requiring supplemental oxygen | 3/33(9.1) | 0 | | | |
| Hospitalised, requiring supplemental oxygen | 1/33(3.0) | 1/30(3.3) | | | |
| Hospitalised, on non-invasive ventilation or high flow oxygen devices | 0 | 1/30(3.3) | | | |
| Death | 5/33(15.2) | 10/30(33.3) | | | |
| Missing | 1/33(3.0) | 0 | | | |
| Baseline CSTAT = 5 | | | | | |
| Not hospitalised, no limitations on activities | 1/7(14.3) | 0 | | | |
| Not hospitalised, limitations on activities | 2/7(28.6) | 6/11(54.5) | | | |
| Hospitalised, not requiring supplemental oxygen | 0 | 1/11(9.1) | | | |
| Death | 4/7(57.1) | 4/11(36.4) | | | |

*Adjusted for minimization variable; CURB score. CSTAT: clinical status based on WHO 7-point ordinal scale.

Symptom duration (days): (a) <10 and (b) ≥10

Table s5 shows the subgroup analysis for duration of pneumonia symptoms. Overall, there was no evidence that the treatment effect was moderated by duration of pneumonia symptoms: interaction effect 0.67 (0.10, 4.36); p-value = 0.582.

**Table s5: Subgroup analysis for duration of symptoms**

| | Placebo N=68 | SFX-01 N=65 | Duration of symptoms | Effect size (99% CI) | p value |
|---|---|---|---|---|---|
| <10 days | | | | | |
| Not hospitalised, no limitations on activities | 5/42(11.9) | 5/45(11.1) | Less than 10 days | 0.82(0.28,2.41) | 0.633 |
| Not hospitalised, limitations on activities | 25/42(59.5) | 26/45(57.8) | 10 days or more | 0.55(0.12,2.54) | 0.314 |
| Hospitalised, not requiring supplemental oxygen | 5/42(11.9) | 1/45(2.2) | Interaction | 0.67(0.10,4.36) | 0.582 |
| Hospitalised, requiring supplemental oxygen | 0 | 1/45(2.2) | | | |
| Hospitalised, on non-invasive ventilation or high flow oxygen devices | 0 | 1/45(2.2) | | | |
| Death | 7/42(16.7) | 11/45(24.4) | | | |
| >= 10 days | | | | | |
| Not hospitalised, no limitations on activities | 6/26(23.1) | 1/20(5.0) | | | |
| Not hospitalised, limitations on activities | 13/26(50.0) | 14/20(70.0) | | | |
| Hospitalised, not requiring supplemental oxygen | 1/26(3.8) | 1/20(5.0) | | | |
| Hospitalised, requiring supplemental oxygen | 1/26(3.8) | 0 | | | |
| Death | 4/26(15.4) | 4/20(20.0) | | | |
| Missing | 1/26(3.8) | 0 | | | |

*Adjusted for minimization variable; CURB score

Other pathogen identified vs no bacterial pathogen identified

Table s6 shows the subgroup analysis for pathogen identified. Overall, there was no evidence that the treatment effect was moderated by whether pathogen was identified: interaction effect 1.68 (0.23, 11.98); p-value = 0.499.

**Table s6: Subgroup analysis for pathogen identified**

| | Placebo N=68 | SFX-01 N=65 | Pathogen identified | Effect size (99% CI) | p value |
|---|---|---|---|---|---|
| No pathogen identified | | | | | |
| Not hospitalised, no limitations on activities | 4/22(18.2) | 3/17(17.6) | Not identified | 0.50(0.09,2.63) | 0.278 |
| Not hospitalised, limitations on activities | 12/22(54.5) | 7/17(41.2) | Identified | 0.83(0.29,2.35) | 0.645 |
| Hospitalised, not requiring supplemental oxygen | 3/22(13.6) | 2/17(11.8) | Interaction | 1.68(0.23,11.98) | 0.499 |
| Hospitalised, requiring supplemental oxygen | 0 | 1/17(5.9) | | | |
| Death | 2/22(9.1) | 4/17(23.5) | | | |
| Missing | 1/22(4.5) | 0 | | | |
| Pathogen identified | | | | | |
| Not hospitalised, no limitations on activities | 7/46(15.2) | 3/48(6.3) | | | |
| Not hospitalised, limitations on activities | 26/46(56.5) | 33/48(68.8) | | | |
| Hospitalised, not requiring supplemental oxygen | 3/46(6.5) | 0 | | | |
| Hospitalised, requiring supplemental oxygen | 1/46(2.2) | 0 | | | |
| Hospitalised, on non-invasive ventilation or high flow oxygen devices | 0 | 1/48(2.1) | | | |
| Death | 9/46(19.6) | 11/48(22.9) | | | |

*Adjusted for minimization variable; CURB score

COVID test: (a) positive and (b) negative

Table s7 shows the subgroup analysis for positive or negative covid test. Overall, there was no evidence that the treatment effect was moderated by covid test: interaction effect 1.94 (0.21, 17.62); p-value = 0.440.

**Table s7: Subgroup analysis for Covid test**

| | Placebo N=68 | SFX-01 N=65 | Covid test | Effect size (99% CI) | p value |
|---|---|---|---|---|---|
| Negative/ Clinically suspected | | | | | |
| Not hospitalised, no limitations on activities | 4/16(25.0) | 3/14(21.4) | Negative | 0.42(0.06,3.07) | 0.265 |
| Not hospitalised, limitations on activities | 8/16(50.0) | 6/14(42.9) | Positive | 0.82(0.31,2.20) | 0.610 |
| Hospitalised, not requiring supplemental oxygen | 2/16(12.5) | 0 | Interaction | 1.94(0.21,17.62) | 0.440 |
| Hospitalised, requiring supplemental oxygen | 0 | 1/14(7.1) | | | |
| Death | 1/16(6.3) | 4/14(28.6) | | | |
| Missing | 1/16(6.3) | 0 | | | |
| Positive | | | | | |
| Not hospitalised, no limitations on activities | 7/52(13.5) | 3/51(5.9) | | | |
| Not hospitalised, limitations on activities | 30/52(57.7) | 34/51(66.7) | | | |
| Hospitalised, not requiring supplemental oxygen | 4/52(7.7) | 2/51(3.9) | | | |
| Hospitalised, requiring supplemental oxygen | 1/52(1.9) | 0 | | | |

| | | |
|---|---|---|
| Hospitalised, on non-invasive ventilation or high flow oxygen devices | 0 | 1/51(2.0) |
| Death | 10/52(19.2) | 11/51(21.6) |

*Adjusted for minimization variable; CURB score

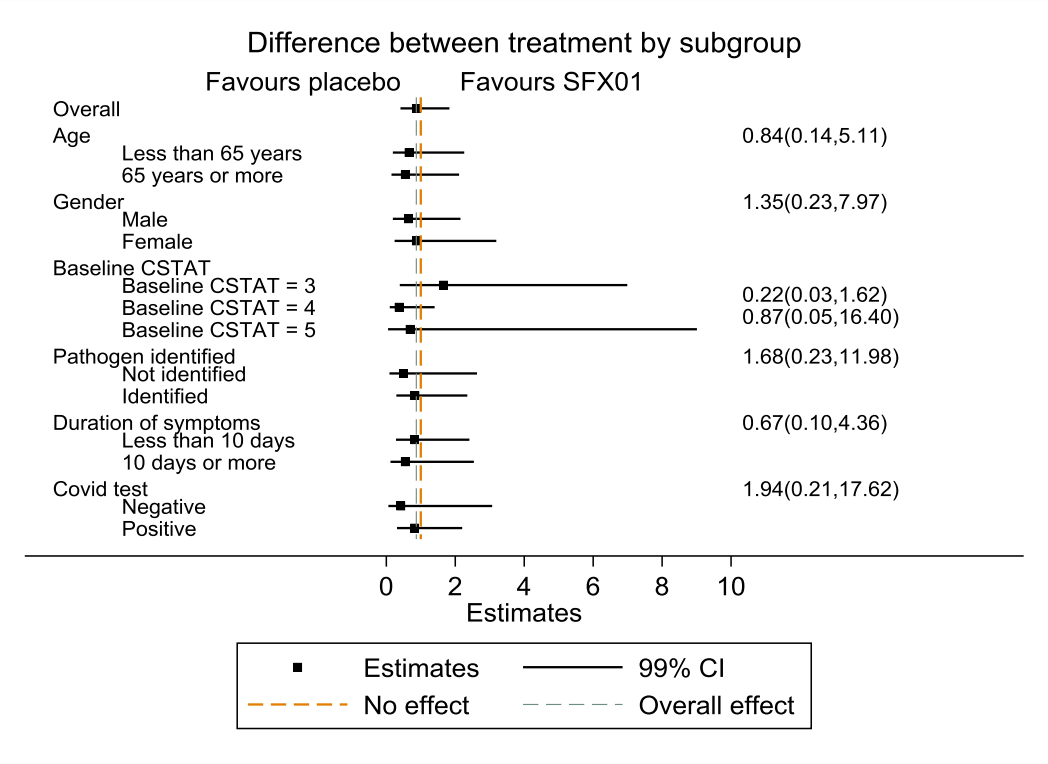


Figure s1: Forest plot for each subgroup.

**Per-protocol analysis**

In total, there were 28 participants (6 in placebo arm and 22 in SFX-01 arm) who discontinued trial drug during the study period. After excluding these participants, 62 participants in the placebo arm and 43 in SFX-01 arm were included in the per-protocol analysis (Table s8). The unadjusted odds ratio (95% confidence interval) from a proportional odds model was 0.57 (0.25, 1.31).

**Table s8: Per-protocol analysis for primary outcome**

| | Placebo N=62 | SFX-01 N=43 | Model | Effect size (95% CI) | p value |
|---|---|---|---|---|---|
| Not hospitalised, no limitations on activities | 3(4.8) | 0 | Unadjusted | 0.57(0.25,1.31) | 0.186 |
| Not hospitalised, limitations on activities | 43(69.4) | 29(67.4) | Adjusted | 0.68(0.29,1.62) | 0.384 |
| Hospitalised, not requiring supplemental oxygen | 3(4.8) | 3(7.0) | | | |
| Hospitalised, requiring supplemental oxygen | 4(6.5) | 1(2.3) | | | |
| Hospitalised, on non-invasive ventilation or high flow oxygen devices | 1(1.6) | 2(4.7) | | | |
| Hospitalised, on invasive mechanical ventilation or ECMO (Extracorporeal membrane oxygenation) | 1(1.6) | 0 | | | |
| Death | 6(9.7) | 8(18.6) | | | |
| Missing | 1(1.6) | 0 | | | |

*Adjusted for minimization variable; CURB score and baseline 7-point ordinal scale
[#]Numbers in cells are n(%) except where indicated.

**Clinical status at day 3, 5, 8, 11, 15 and 29**

The number and percentage of participants in each clinical status category for day 3, 5, 8, 11, 15 and 29 are summarized and reported in Table s9.

**Table s9: Clinical status at day 3, 5, 8, 11, 15 and 29**

| | | Placebo N=68 | SFX-01 N=65 | Total N=133 |
|---|---|---|---|---|
| Day 3 | Not hospitalised, limitations on activities | 12(17.6%) | 17(26.2%) | 29(21.8%) |
| | Hospitalised, not requiring supplemental oxygen | 22(32.4%) | 8(12.3%) | 30(22.6%) |
| | Hospitalised, requiring supplemental oxygen | 23(33.8%) | 18(27.7%) | 41(30.8%) |
| | Hospitalised, on non-invasive ventilation or high flow oxygen devices | 7(10.3%) | 16(24.6%) | 23(17.3%) |
| | Hospitalised, on invasive mechanical ventilation or ECMO (Extracorporeal membrane oxygenation) | 1(1.5%) | | 1(0.8%) |
| | Death | 2(2.9%) | 2(3.1%) | 4(3.0%) |
| | Missing | 1(1.5%) | 4(6.2%) | 5(3.8%) |
| Day 5 | Not hospitalised, limitations on activities | 26(38.2%) | 29(44.6%) | 55(41.4%) |
| | Hospitalised, not requiring supplemental oxygen | 15(22.1%) | 6(9.2%) | 21(15.8%) |
| | Hospitalised, requiring supplemental oxygen | 15(22.1%) | 11(16.9%) | 26(19.5%) |
| | Hospitalised, on non-invasive ventilation or high flow oxygen devices | 7(10.3%) | 12(18.5%) | 19(14.3%) |
| | Hospitalised, on invasive mechanical ventilation or ECMO (Extracorporeal membrane oxygenation) | 1(1.5%) | | 1(0.8%) |
| | Death | 3(4.4%) | 4(6.2%) | 7(5.3%) |
| | Missing | 1(1.5%) | 3(4.6%) | 4(3.0%) |
| Day 8 | Not hospitalised, no limitations on activities | 1(1.5%) | | 1(0.8%) |
| | Not hospitalised, limitations on activities | 36(52.9%) | 38(58.5%) | 74(55.6%) |
| | Hospitalised, not requiring supplemental oxygen | 7(10.3%) | 6(9.2%) | 13(9.8%) |
| | Hospitalised, requiring supplemental oxygen | 12(17.6%) | 6(9.2%) | 18(13.5%) |
| | Hospitalised, on non-invasive ventilation or high flow oxygen devices | 6(8.8%) | 7(10.8%) | 13(9.8%) |
| | Hospitalised, on invasive mechanical ventilation or ECMO (Extracorporeal | 2(2.9%) | 1(1.5%) | 3(2.3%) |

| | | | | |
|---|---|---|---|---|
| | membrane oxygenation) | | | |
| | Death | 3(4.4%) | 6(9.2%) | 9(6.8%) |
| | Missing | 1(1.5%) | 1(1.5%) | 2(1.5%) |
| Day 11 | Not hospitalised, no limitations on activities | 1(1.5%) | | 1(0.8%) |
| | Not hospitalised, limitations on activities | 44(64.7%) | 42(64.6%) | 86(64.7%) |
| | Hospitalised, not requiring supplemental oxygen | 5(7.4%) | 7(10.8%) | 12(9.0%) |
| | Hospitalised, requiring supplemental oxygen | 6(8.8%) | 2(3.1%) | 8(6.0%) |
| | Hospitalised, on non-invasive ventilation or high flow oxygen devices | 4(5.9%) | 4(6.2%) | 8(6.0%) |
| | Hospitalised, on invasive mechanical ventilation or ECMO (Extracorporeal membrane oxygenation) | 1(1.5%) | 2(3.1%) | 3(2.3%) |
| | Death | 6(8.8%) | 7(10.8%) | 13(9.8%) |
| | Missing | 1(1.5%) | 1(1.5%) | 2(1.5%) |
| Day 15 | Not hospitalised, no limitations on activities | 3(4.4%) | | 3(2.3%) |
| | Not hospitalised, limitations on activities | 44(64.7%) | 46(70.8%) | 90(67.7%) |
| | Hospitalised, not requiring supplemental oxygen | 5(7.4%) | 4(6.2%) | 9(6.8%) |
| | Hospitalised, requiring supplemental oxygen | 4(5.9%) | 1(1.5%) | 5(3.8%) |
| | Hospitalised, on non-invasive ventilation or high flow oxygen devices | 2(2.9%) | 3(4.6%) | 5(3.8%) |
| | Hospitalised, on invasive mechanical ventilation or ECMO (Extracorporeal membrane oxygenation) | 1(1.5%) | | 1(0.8%) |
| | Death | 8(11.8%) | 11(16.9%) | 19(14.3%) |
| | Missing | 1(1.5%) | | 1(0.8%) |
| Day 29 | Not hospitalised, no limitations on activities | 11(16.2%) | 6(9.2%) | 17(12.8%) |
| | Not hospitalised, limitations on activities | 38(55.9%) | 40(61.5%) | 78(58.6%) |
| | Hospitalised, not requiring supplemental oxygen | 6(8.8%) | 2(3.1%) | 8(6.0%) |
| | Hospitalised, requiring supplemental | 1(1.5%) | 1(1.5%) | 2(1.5%) |

| | | | |
|---|---|---|---|
| oxygen | | | |
| Hospitalised, on non-invasive ventilation or high flow oxygen devices | | 1(1.5%) | 1(0.8%) |
| Death | 11(16.2%) | 15(23.1%) | 26(19.5%) |
| Missing | 1(1.5%) | | 1(0.8%) |

*Numbers in cells are n(%)

**Discontinuation of treatment**

Of 133 participants, 28 (21.1%); 6 (8.8%) in placebo arm and 22 (33.8%) in SFX-01 arm discontinued the trial treatment during the study (IRR: 3.79 (95% CI 1.53, 9.34); p=0.004). The main reasons for discontinuation were due to adverse events and participant's choice (Table s10).

**Table s10: Discontinuation of treatment**

| | Placebo N=68 | SFX-01 N=65 | Total N=133 |
|---|---|---|---|
| Number of participants who continued the treatment throughout the study | 62(91.2) | 43(66.2) | 105 (78.9) |
| Number of participants who discontinued the treatment | 6(8.8) | 22(33.8) | 28 (21.1) |
| Reason for discontinuation of treatment | | | |
| Advice from GP/other healthcare professional | 1(16.7) | 0 | 1(3.6) |
| Other Adverse event | 2(33.3) | 10(45.5) | 12(42.9) |
| Participant's choice | 2(33.3) | 10(45.5) | 12(42.9) |
| Other | 1(16.7) | 1(4.5) | 2(7.1) |
| Missing | 0 | 1(4.5) | 1(3.6) |

*Numbers in cells are n(%) except where indicated.

**Adverse events, system organ class (SOC) and preferred term level**

Tables s11 and s12 show the adverse events reported during the study. Overall, 72 participants; 30 (44.1%) in placebo arm and 42 (64.6%) in SFX-01 arm reported at least one adverse event (IRR: 1.48 (95% CI 0.92, 2.36); p=0.103). There were in total, 97 events; 42 in

placebo and 55 in SFX-01. The most common adverse events were gastrointestinal disorders and infections and infestations.

**Table s11: Adverse events and system organ class**

| | | Placebo N=68 | SFX-01 N=65 | Total N=133 |
|---|---|---|---|---|
| Number of participants with no adverse events | | 38(55.9) | 23(35.4) | 61(45.9) |
| Number of participants with adverse events | | 30(44.1) | 42(64.6) | 72(54.1) |
| Number of adverse events | | 42 | 55 | 97 |
| Severity | Mild | 13(31.0) | 35(63.6) | 48(49.5) |
| | Moderate | 11(26.2) | 4(7.3) | 15(15.5) |
| | Severe | 18(42.9) | 16(29.1) | 34(35.1) |
| System Organ Class Level | | | | |
| | Cardiac disorders | 2(4.8) | 1(1.8) | 3(3.1) |
| | Eye disorders | 1(2.4) | 0 | 1(1.0) |
| | Gastrointestinal disorders | 10(23.8) | 33(60.0) | 43(44.3) |
| | General disorders and administration site conditions | 2(4.8) | 1(1.8) | 3(3.1) |
| | Infections and infestations | 15(35.7) | 15(27.3) | 30(30.9) |
| | Metabolism and nutrition disorders | 1(2.4) | 0 | 1(1.0) |
| | Musculoskeletal and connective tissue disorders | 1(2.4) | 0 | 1(1.0) |
| | Neoplasms benign, malignant and unspecified (incl cysts and polyps) | 1(2.4) | 1(1.8) | 2(2.1) |
| | Nervous system disorders | 2(4.8) | 2(3.6) | 4(4.1) |
| | Renal and urinary disorders | 2(4.8) | 0 | 2(2.1) |
| | Respiratory, thoracic and mediastinal disorders | 4(9.5) | 2(3.6) | 6(6.2) |
| | Skin and subcutaneous tissue disorders | 1(2.4) | 0 | 1(1.0) |

*Numbers in cells are n(%)

**Table s12: Summary of all adverse events – Preferred Term Level**

| | Placebo N=68 | SFX-01 N=65 | Total N=133 |
|---|---|---|---|
| Number of participants with no adverse events | 38(55.9) | 23(35.4) | 61(45.9) |
| Number of participants with adverse events | 30(44.1) | 42(64.6) | 72(54.1) |
| Preferred Term Level | | | |
| Abdominal discomfort | 0 | 1(1.8) | 1(1.0) |
| Abdominal pain | 1(2.4) | 4(7.3) | 5(5.2) |
| Abdominal pain upper | 0 | 5(9.1) | 5(5.2) |
| Acute kidney injury | 1(2.4) | 0 | 1(1.0) |
| Acute myocardial infarction | 0 | 1(1.8) | 1(1.0) |
| COVID-19 | 11(26.2) | 10(18.2) | 21(21.6) |
| Cardiac failure | 1(2.4) | 0 | 1(1.0) |
| Cerebrovascular accident | 1(2.4) | 0 | 1(1.0) |
| Chest pain | 1(2.4) | 1(1.8) | 2(2.1) |
| Diarrhoea | 6(14.3) | 3(5.5) | 9(9.3) |
| Dizziness | 0 | 1(1.8) | 1(1.0) |
| Dry throat | 0 | 1(1.8) | 1(1.0) |
| Dyspepsia | 0 | 9(16.4) | 9(9.3) |
| Empyema | 1(2.4) | 0 | 1(1.0) |
| Eye pain | 1(2.4) | 0 | 1(1.0) |
| Furuncle | 0 | 1(1.8) | 1(1.0) |
| Haemorrhoids | 1(2.4) | 0 | 1(1.0) |
| Interstitial lung disease | 0 | 1(1.8) | 1(1.0) |
| Intestinal perforation | 1(2.4) | 0 | 1(1.0) |
| Ischaemic stroke | 1(2.4) | 0 | 1(1.0) |
| Lower respiratory tract infection | 1(2.4) | 0 | 1(1.0) |
| Lung adenocarcinoma | 0 | 1(1.8) | 1(1.0) |
| Musculoskeletal stiffness | 1(2.4) | 0 | 1(1.0) |
| Nausea | 0 | 5(9.1) | 5(5.2) |
| Oedema peripheral | 1(2.4) | 0 | 1(1.0) |
| Pericardial effusion | 1(2.4) | 0 | 1(1.0) |
| Pharyngitis | 1(2.4) | 0 | 1(1.0) |
| Pneumonia | 0 | 2(3.6) | 2(2.1) |
| Pollakiuria | 1(2.4) | 0 | 1(1.0) |
| Pulmonary embolism | 2(4.8) | 0 | 2(2.1) |
| Pulmonary fibrosis | 1(2.4) | 0 | 1(1.0) |
| Rash | 1(2.4) | 0 | 1(1.0) |
| Seizure | 0 | 1(1.8) | 1(1.0) |
| Type 2 diabetes mellitus | 1(2.4) | 0 | 1(1.0) |
| Urinary tract infection | 1(2.4) | 2(3.6) | 3(3.1) |

| | | | |
|---|---|---|---|
| Vomiting | 1(2.4) | 6(10.9) | 7(7.2) |
| Vulval cancer metastatic | 1(2.4) | 0 | 1(1.0) |
| Wheezing | 1(2.4) | 0 | 1(1.0) |

*Numbers in cells are n(%)

Table s13 shows the details of the adverse events. Of 97 events reported, 16 (38.1%) in placebo arm and 19 (34.5%) were possibly due to the treatment received and 18 (42.9%) and 34(61.8%) in placebo and SFX-01 arms recovered, respectively.

**Table s13: Details of adverse events**

| | Placebo N=68 | SFX-01 N=65 | Total N=133 |
|---|---|---|---|
| Number of participants with no adverse events | 38(55.9) | 23(35.4) | 61 (45.9) |
| Number of participants with adverse events | 30(44.1) | 42(64.6) | 72 (54.1) |
| Number of adverse events | 42 | 55 | 97 |
| Action Taken | | | |
| None | 18(42.9) | 27(49.1) | 45(46.4) |
| Hospitalisation | 5(11.9) | 1(1.8) | 6(6.2) |
| IMP temporarily stopped | 0 | 1(1.8) | 1(1.0) |
| IMP permanently stopped | 4(9.5) | 20(36.4) | 24(24.7) |
| Con meds commenced | 9(21.4) | 3(5.5) | 12(12.4) |
| Others | 4(9.5) | 3(5.5) | 7(7.2) |
| Hospitalisation & Other | 1(2.4) | 0 | 1(1.0) |
| IMP permanently stopped & Con meds commenced | 1(2.4) | 0 | 1(1.0) |
| Relationship to trial drug | | | |
| None | 26(61.9) | 22(40.0) | 48(49.5) |
| Possible | 16(38.1) | 19(34.5) | 35(36.1) |
| Probable | 0 | 14(25.5) | 14(14.4) |
| Outcome | | | |
| Recovered | 18(42.9) | 34(61.8) | 52(53.6) |
| Recovered with sequelae | 4(9.5) | 2(3.6) | 6(6.2) |
| Recovering | 6(14.3) | 0 | 6(6.2) |
| Not recovered | 3(7.1) | 0 | 3(3.1) |
| Unknown | 0 | 4(7.3) | 4(4.1) |
| Fatal | 11(26.2) | 15(27.3) | 26(26.8) |

*Numbers in cells are n(%)

**Serious adverse events and system organ class level**

Of 133 participants, 34 (25.6%) had at least one serious adverse event; 18 (26.5%) in placebo arm and 16 (24.6%) in SFX-01 arm. There were 35 SAEs in total; 19 in placebo arm and 16 in SFX-01 arm. The most common serious adverse events were infections and infestations (Table s14).

**Table s14: Serious adverse events**

| | Placebo N=68 | SFX-01 N=65 | Total N=133 |
|---|---|---|---|
| Number of participants with no serious adverse events | 50(73.5) | 49(75.4) | 99 (74.4) |
| Number of participants with serious adverse events | 18(26.5) | 16(24.6) | 34 (25.6) |
| Number of serious adverse events | 19 | 16 | 35 |
| System Organ Class Level | | | |
| Cardiac disorders | 2(10.5) | 1(6.3) | 3 (8.6) |
| Gastrointestinal disorders | 1(5.3) | 0 | 1 (2.9) |
| Infections and infestations | 12(63.2) | 12(75.0) | 24 (68.6) |
| Neoplasms benign, malignant and unspecified (incl cysts and polyps) | 1(5.3) | 1(6.3) | 2 (5.7) |
| Nervous system disorders | 2(10.5) | 1(6.3) | 3 (8.6) |
| Renal and urinary disorders | 1(5.3) | 0 | 1 (2.9) |
| Respiratory, thoracic and mediastinal disorders | 0 | 1(6.3) | 1 (2.9) |

*Numbers in cells are n(%)

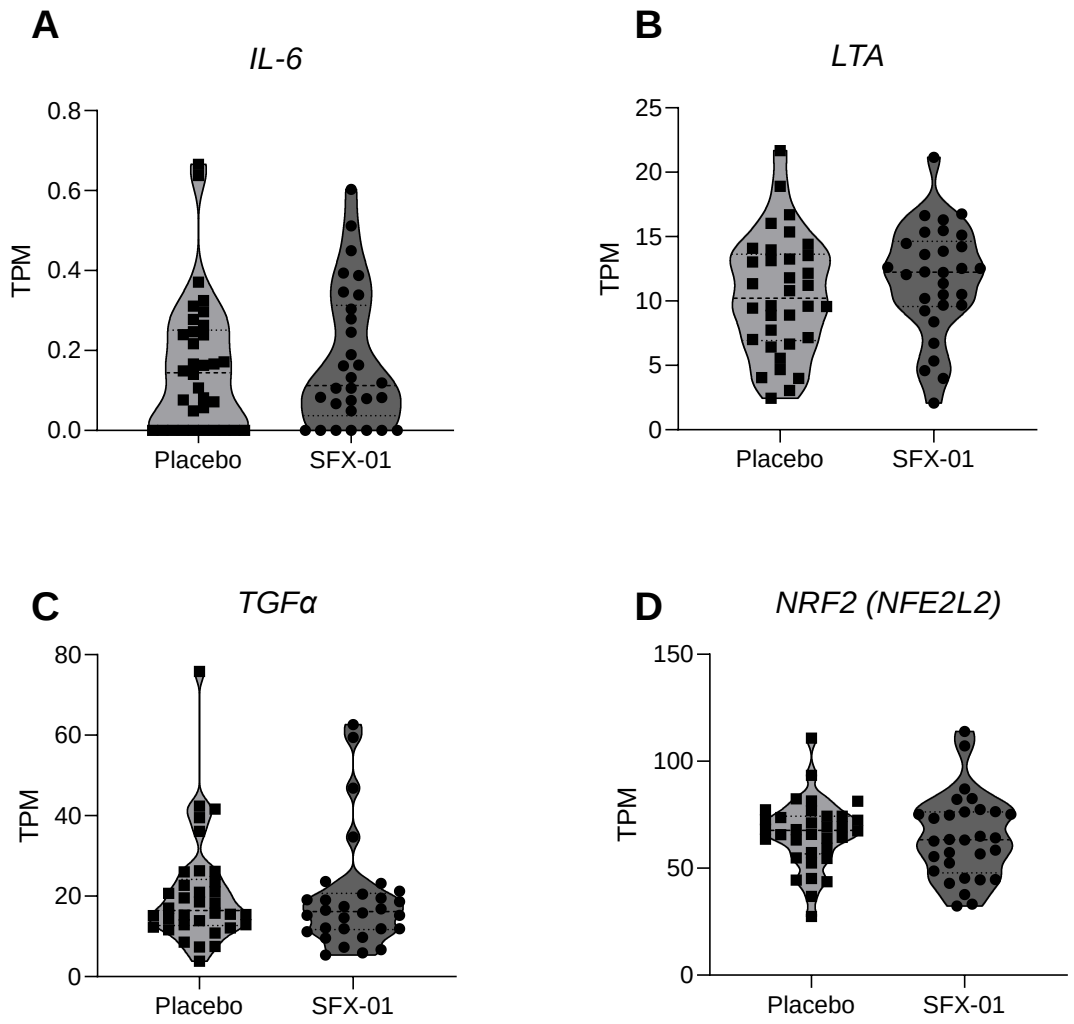


**Figure s2. Peripheral blood leukocyte gene expression in individuals receiving SFX-01 or placebo treatment at day 15**

Peripheral blood leukocyte gene expression analysed by mRNA sequencing was compared between SFX-01 and placebo treated individuals who completed 14 days of trial treatment. Individual transcript per million (TPM) values representing relative expression levels of genes of interest: (A) Interleukin-6 (*IL-6*), (B) Lymphotoxin-alpha (*LTA*), (C) transforming growth factor-alpha (*TGFα*) and Nrf2 encoded by the *NFE2L2* gene. Day 15: SFX-01 n=30, placebo n=34.

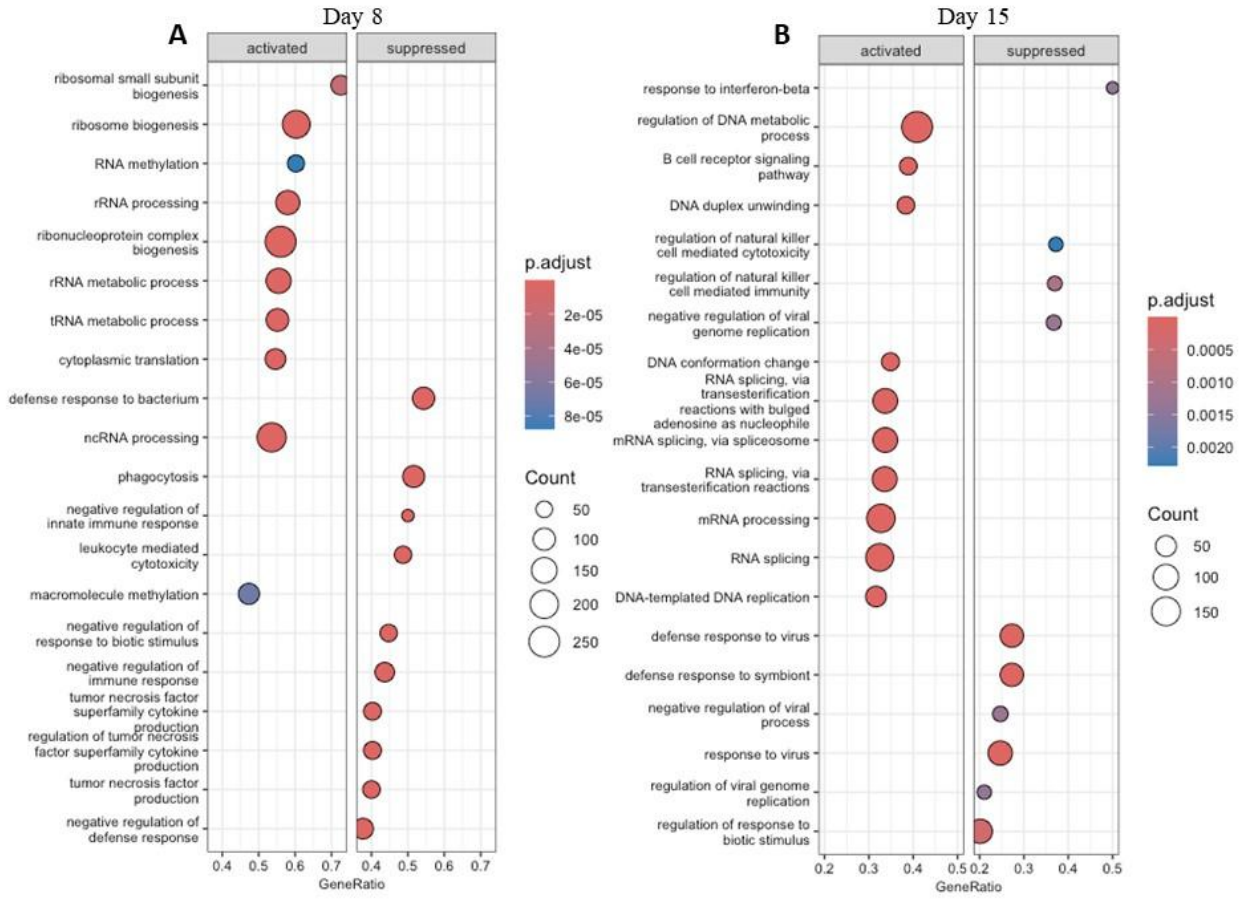


**Figure s3. Gene set enrichment analysis (GSEA) comparing participants receiving SFX-01 vs. placebo.**

GSEA was performed for gene expression data at day 8 (A) and day 15 (B). Top 10 biological pathways identified as positively (activated) and negatively (supressed) enriched in those receiving SFX-01 compared to those receiving placebo are shown. Dot size indicates the number of genes associated with the pathway. Similar  Processes are ranked by decreasing gene ratio (i.e. proportion of genes associated with treatment group out of the total number of genes in a given pathway). To rank genes for GSEA, the signed -log10(p-value) was generated from the p-value and the sign of the Log2 fold change from differential expression analysis of drug vs. placebo groups. Day 8: SFX-01 N=14, placebo N=18; day 15: SFX-01 N=30, placebo N=34.

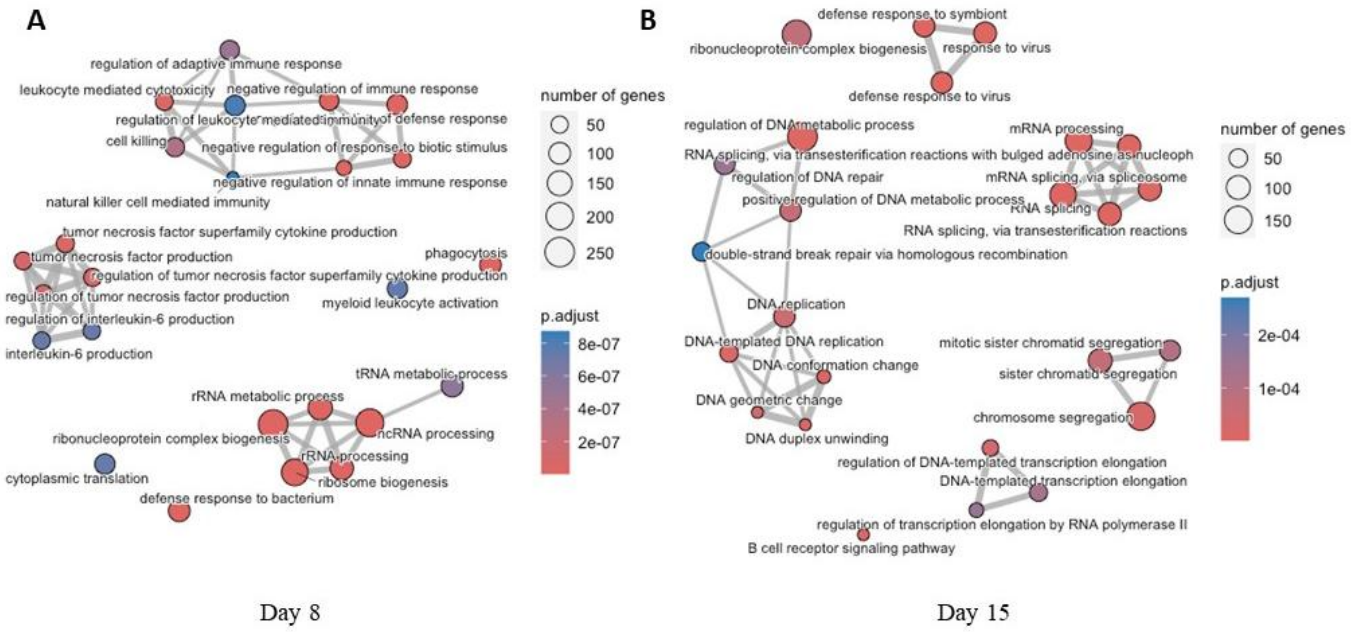


**Figure s4. Pathway clustering after gene set enrichment analysis (GSEA) comparing participants receiving SFX-01 vs. placebo.**

Clustering of the top 25 pathways identified between the SFX-01 and placebo groups by GSEA was performed for gene expression data at day 8 (A) and day 15 (B). Dot size indicates the number of genes associated with the pathway. Pathways joined by lines/edges are similar, edge length and thickness are proportional to term similarity. Day 8: SFX-01 N=14, placebo N=18; day 15: SFX-01 N=30, placebo N=34.

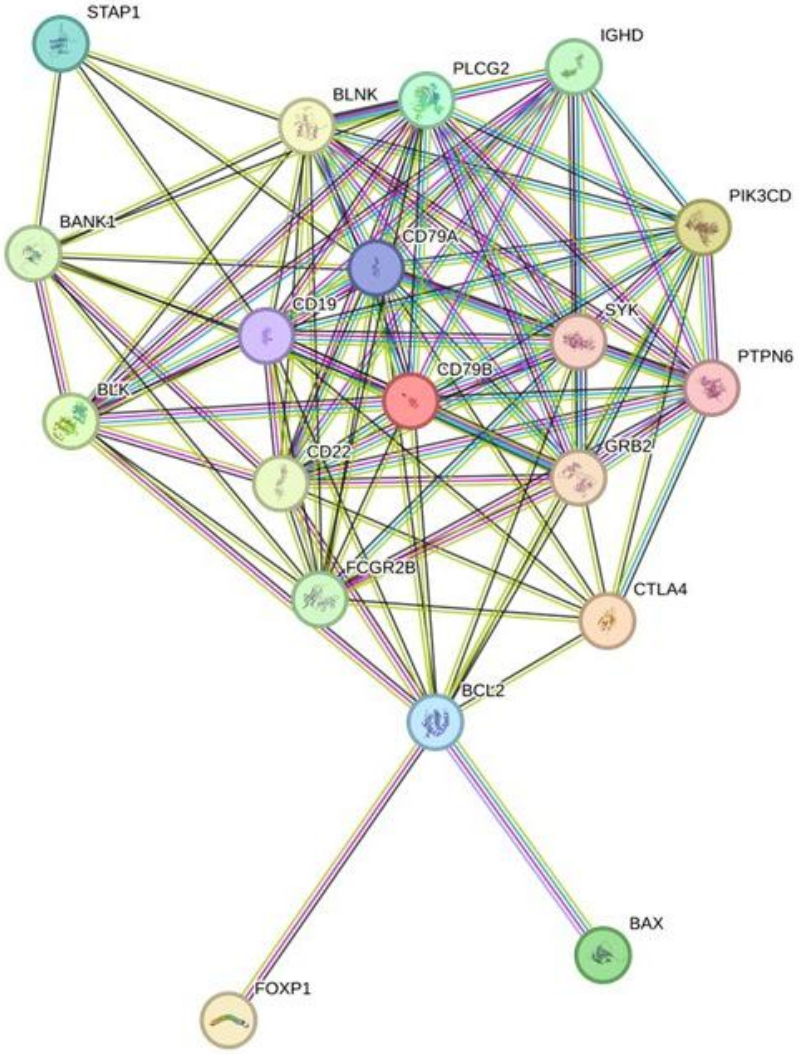


**Figure s5. Protein–protein interaction network for genes identified in the B cell receptor signalling pathway upregulated in the SFX-01 group at day 15.**

Edges represent protein – protein associations, edge colours represent type of interaction and information source. Key: known interaction: teal- from curated database, purple- experimentally determined. Predicted interaction: red- gene fusions, blue- gene co-occurrence. Other: green- identified by textmining, black- co-expression, lilac- protein homology. 19 of the 28 differential genes identified in this pathway are displayed. Day 15: SFX-01 N=30, placebo N=34.