

The Effect of Inhaled Interferon Beta-1a (SNG001) Treatment Compared to Placebo on Lung Antiviral Biomarkers and Viral Clearance in COPD Patients with Respiratory Virus Infections

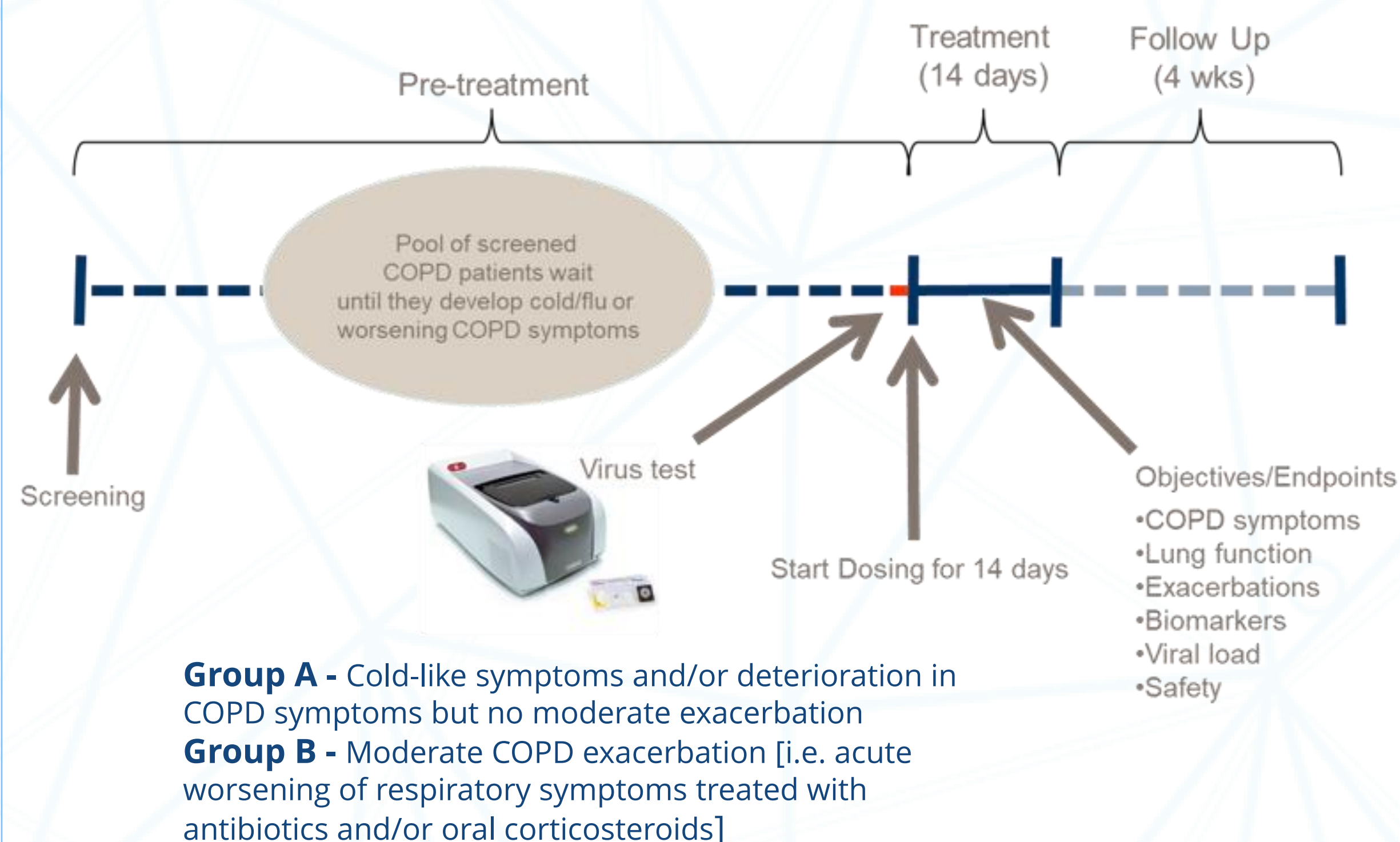
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P. Monk¹, J. Brookes¹, V. Tear¹, T. Batten², C. Newall², M. Mankowski³, M. Crooks⁴, D. Singh⁵, R. Chaudhuri⁶, K. Lunn¹, S. Reynolds¹, S. Dudley¹, F. Gabbay³, S. Holgate⁷, R. Djukanovic⁷ & T. Wilkinson⁷

¹ Synairgen Research Ltd., UK, ² Veramed, UK, ³ TranScrip, UK, ⁴ University of Hull, UK, ⁵ University of Manchester, UK, ⁶ University of Glasgow, UK, ⁷ University of Southampton, UK.

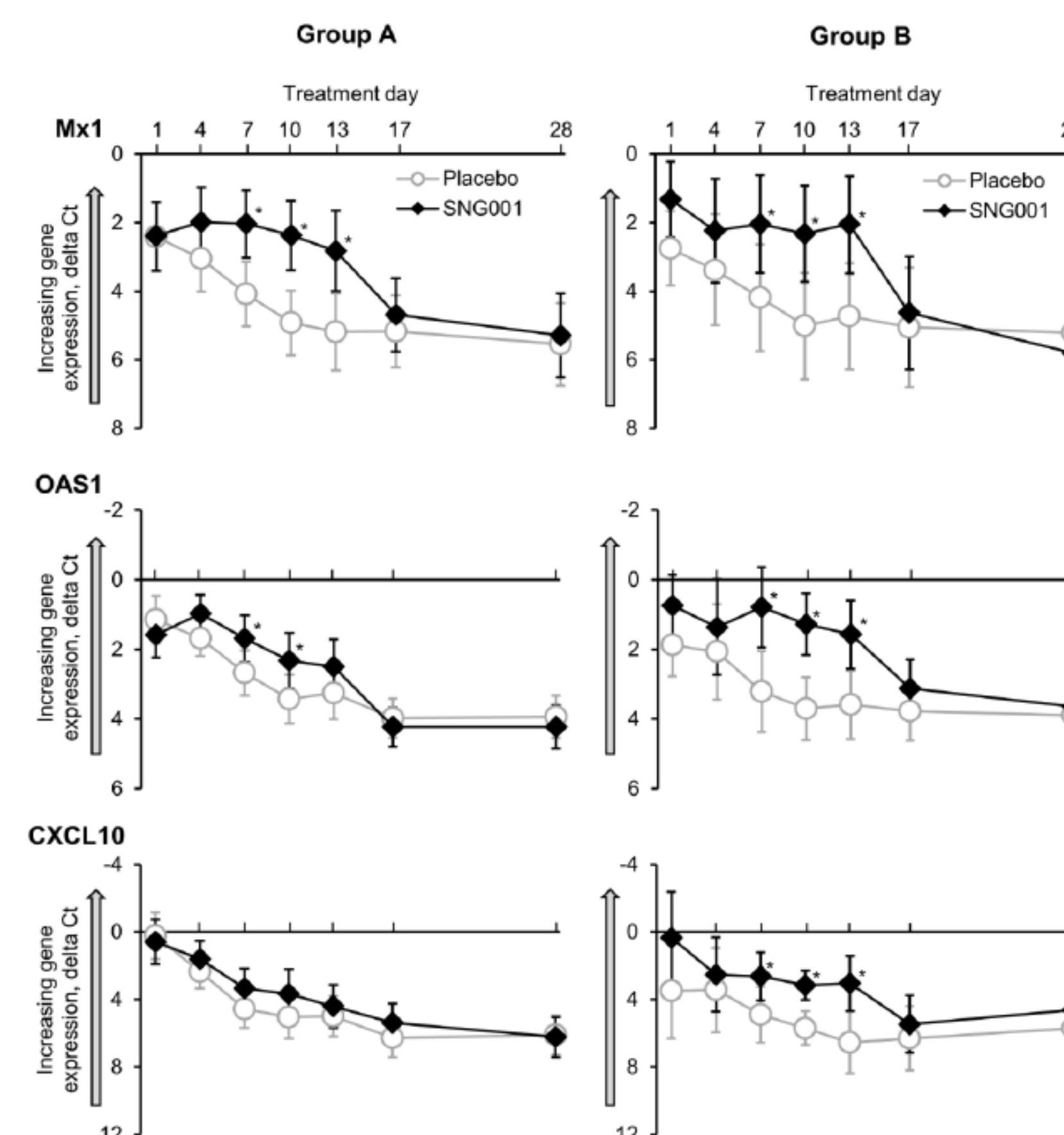
Rationale for and Design of the SG015 Clinical Trial (NCT03570359): A Randomized, Placebo-Controlled Phase 2 Clinical Study of Inhaled Interferon beta (SNG001) Conducted in COPD Patients with a Confirmed Respiratory Viral Infection

- Respiratory viral infections (RVIs) are major drivers of chronic obstructive pulmonary disease (COPD) exacerbations when upper respiratory tract infections spread to the lungs to worsen pre-existing inflammation.
- Interferon beta (IFN- β) is key in host defense against viruses driving the expression of hundreds of so-called interferon stimulated genes (ISGs) that encode antiviral proteins that act across the viral replication cycle conferring broad spectrum antiviral activity.
- Correspondingly, IFN- β has been shown to have antiviral activity against a broad range of respiratory viruses in cell-based assays including human rhinovirus (HRV), influenza, respiratory syncytial virus (RSV) and SARS-CoV-2.
- Deficient or delayed IFN- β responses in the lungs of COPD patients may explain why COPD patients are at increased risk of developing more severe viral lung infections providing the rationale for developing our formulation of IFN- β for inhalation, SNG001, as a potential treatment for virus-induced exacerbations of COPD (García-Valero *et al.* PLoS One. 2019;14(6):e0217803; Veerati *et al.*, Front Immunol. 2020;11:974).
- SG015 was a randomized, placebo-controlled Phase 2 clinical study of inhaled SNG001 conducted in COPD patients with a confirmed RVI (refer to schematic below). 109 COPD patients with worsening symptoms and a positive respiratory virus test (BioFire® Respiratory panel) were randomized 1:1 to SNG001 or placebo once-daily for 14 days in two Groups: Group A (cold-like symptoms and/or deterioration in COPD symptoms but no moderate exacerbation; Placebo n=36, SNG001 n=38); Group B (moderate COPD exacerbation [i.e. acute worsening of respiratory symptoms treated with antibiotics and/or oral corticosteroids], Placebo n=16, SNG001 n=19).
- Sputum samples were collected on days 1, 4, 7, 10, 13, 17 and 28 for analysis of lung antiviral biomarker responses and viral clearance from the lungs



Upregulation of Lung Antiviral Biomarkers

- To assess lung antiviral responses to SNG001 treatment compared to placebo, sputum samples were obtained around 24 hours post dose over the treatment period and in the follow up period, and sputum cell gene expression of interferon stimulated genes (ISGs), namely MX1, OAS1 and CXCL10, assessed by RT-qPCR.
- The results show that SNG001 upregulated lung antiviral defenses with antiviral responses returning to that of placebo by Day 17 after patients had received their last dose on Day 14 (see below):
 - Mx1 and OAS1 sputum cell gene expression was significantly upregulated on Day 7, 10 and 13 ($p < 0.05$) overall and in Groups A and B with SNG001 treatment compared to placebo.
 - CXCL10 sputum cell gene expression was significantly upregulated in the overall population with SNG001 treatment compared to placebo on Days 7 and 10, in Group B on Days 7, 10 and 13, and there was no significant difference in Group A.

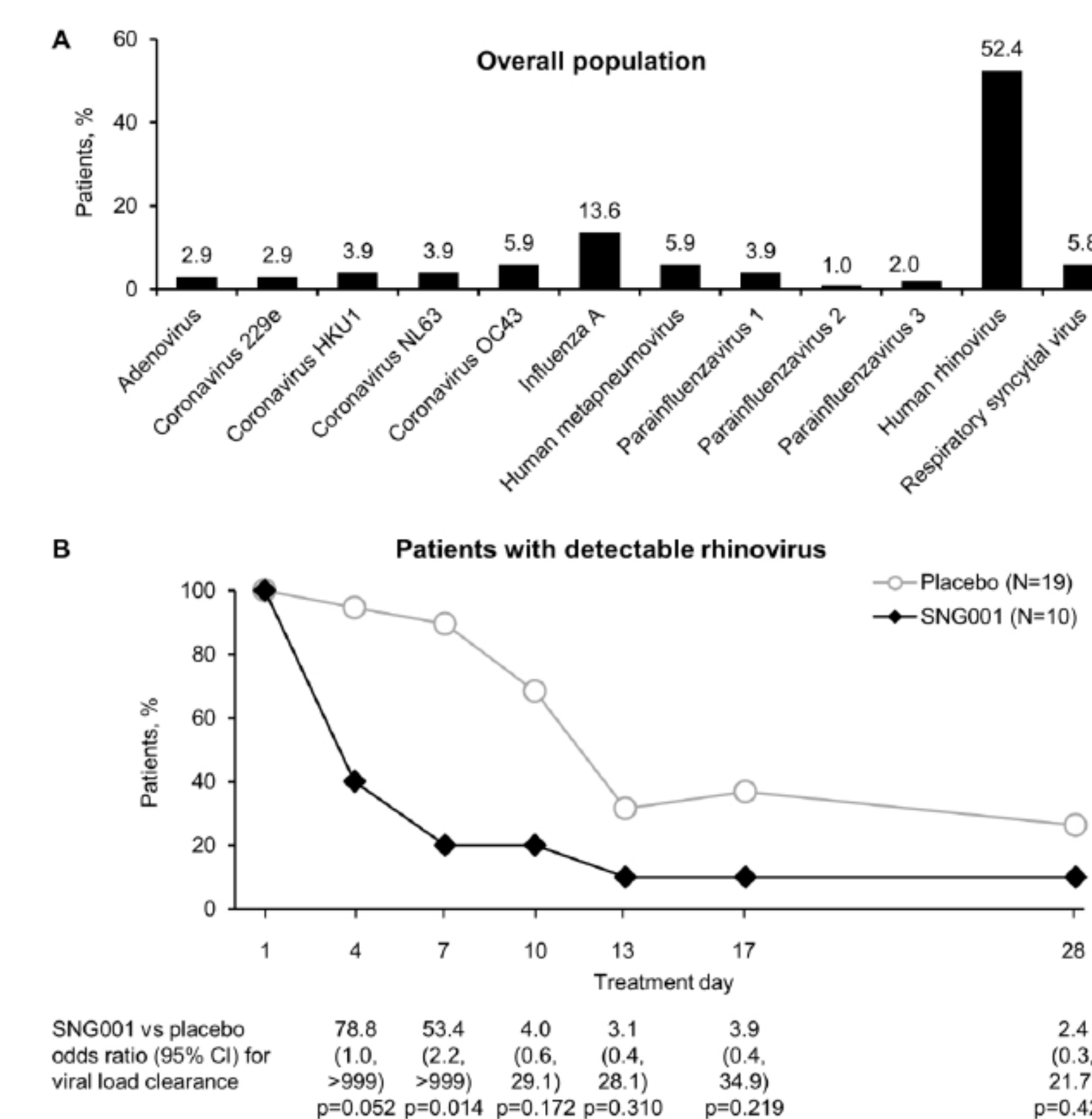


Sputum cell interferon-stimulated gene expression

Data are plotted normalized to housekeeping (reference) genes and are least squares mean and 95% confidence interval (* $p < 0.05$ SNG001 vs placebo). Group A: n=18 placebo, n=19 SNG001. Group B: n=8-9 placebo, n=7 SNG001.

Accelerated Viral (HRV) Clearance from the lungs

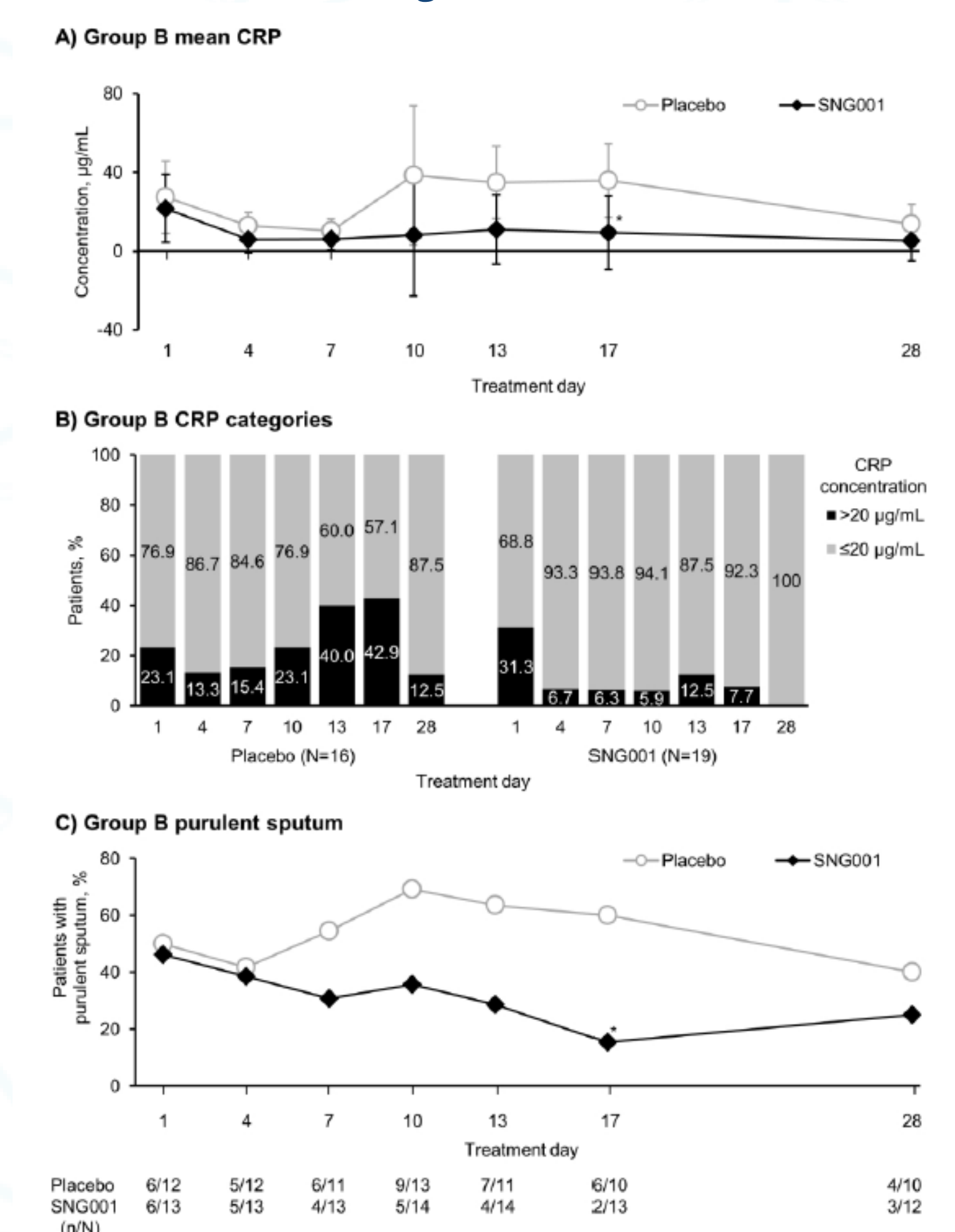
- Patients entering the study had a broad range of respiratory viral infections with, as anticipated, the most common virus detected being HRV (52% of patients; Figure A). The next most detected virus was influenza (13.6%).
- To assess viral clearance from the lungs in response to SNG001 treatment compared to placebo, sputum samples were obtained over the treatment and follow up periods and the presence of viruses assessed by RT-qPCR.
- As HRV was by far the most common virus, a *post hoc* analysis of viral clearance was conducted in the subgroup of patients who had detectable HRV in sputum at baseline.
- By Day 4 the proportion of patients who had detectable rhinovirus had fallen to 40.0% in patients receiving SNG001 compared to 94.7% in patients receiving placebo ($p = 0.052$) and by Day 7 to 20.0% in patients receiving SNG001 compared to 89.5% in patients receiving placebo ($p = 0.014$), suggesting SNG001 had accelerated viral clearance from the lungs (Figure B).



(A) Summary of baseline positive respiratory virus test results in the overall population; (B) Proportion of patients with detectable viral load for rhinovirus at baseline who had detectable rhinovirus viral load at each visit. N values are the number of patients with detectable viral load for rhinovirus at baseline.

Impact on secondary bacterial infections?

- Group B patients receiving placebo had an increase in serum CRP concentration in the second week of treatment (Days 10-17) which was not seen with SNG001 (Figure A).
- A *post hoc* analysis was conducted in Group B, with CRP levels categorised by a cut point of 20 $\mu\text{g/mL}$ at each visit. With placebo but not SNG001, an increasing proportion of patients had CRP > 20 $\mu\text{g/mL}$ from Day 4 to Day 17 (Figure B).
- Increases in CRP were associated with increased incidence of purulent sputum (Figure C) in the placebo group suggesting that SNG001 treatment may have had an impact on the development of secondary bacterial infections. On Day 17, 60.0% of the patients treated with placebo had purulent sputum compared to 15.4% of patients treated with SNG001 ($p = 0.039$). This potential impact of SNG001 treatment on the emergence of secondary bacterial infections would need to be confirmed in a future clinical trial assessing bacterial load.



A) Mean and B) categorical analyses of serum CRP, and C) proportion of patients with purulent sputum in Group B. * $p < 0.05$ SNG001 vs placebo. Panel A: Data are least squares mean and 95% confidence intervals, n=13 placebo, n=16 SNG001. Panel C: Sputum graded on colour scale from 1 to 5; with Grades of 3 to 5 reflecting 'purulent' sputum.

Statistical analysis

- Exploratory analyses of sputum antiviral-stimulated genes, viral load clearance and sputum purulence were conducted following unblinding of the study data. Sputum antiviral-stimulated genes were analysed over time using a MMRM with treatment, visit and the treatment by visit interaction as covariates. The models were fitted with an unstructured variance-covariance matrix. Analyses were conducted separately for Group A and Group B.
- Viral load clearance for patients with detectable rhinovirus was analysed at each post-baseline visit using a Firth logistic regression model with covariates for treatment and baseline viral load. Viral load clearance was defined as undetectable rhinovirus viral load.
- Sputum purulence, defined as sputum colour classified as 3, 4 or 5 according to the BronkoTest colour chart was analysed at each visit using Fisher's exact test. Analyses were conducted separately for Group A and Group B.

Acknowledgements

We would like to thank all the patients who have taken part in our clinical trials and the study teams who conducted them.

Disclosures

Synairgen is a UK-based biotech company developing inhaled interferon beta (SNG001) as a potential treatment for severe viral lung infections (www.Synairgen.com).

Conclusions

Inhaled SNG001 treatment upregulated lung antiviral defenses as assessed using sputum cell biomarker responses and was associated with accelerated viral clearance, supporting the proposed mechanism of action as an antiviral treatment for severe viral lung infections.