Effect of SNG001, Inhaled Interferon-β1a, on SARS-CoV-2 Diversity and Evolution

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BACKGROUND

- SARS-CoV-2 can suppress the host interferon (IFN) response, a critical component of innate immunity.
- SNG001 is an inhaled IFN-β1a treatment, given for 14 days, for COVID-19 evaluated in the ACTIV-2/A5401 trial.
- Although it did not accelerate nasal viral RNA decay, there was a trend towards fewer hospitalizations in the treated arm.
- The impact of exogenous IFN treatment on viral genomes is unknown.
- We investigated SARS-CoV-2 evolution and diversity in response to inhaled SNG001 in the ACTIV-2 study.

ACTIV-2/A5401 STUDY DESIGN

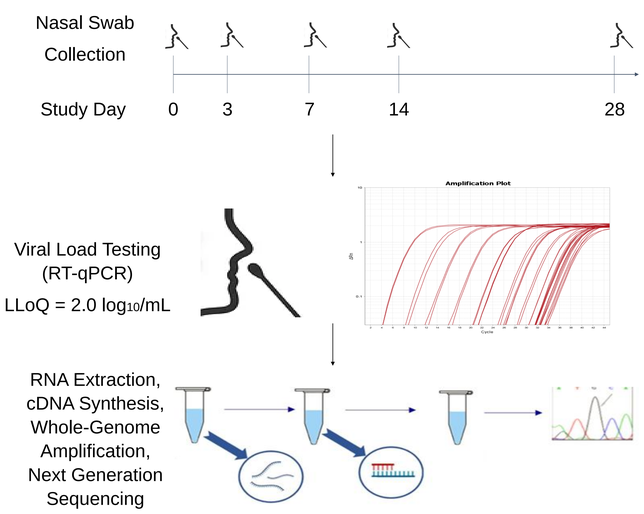
Key Inclusion Criteria

- Adult outpatient with confirmed COVID-19
- Study entry ≤10 days of diagnosis or symptom onset

Study Population

- 220 outpatients randomized to the SNG001 and Placebo groups (n=110 for each)
- Enrolled at 25 U.S. sites during February - August 2021

Sample Collection & Laboratory Assays



Viral Sequence Analysis

- Viral sequence diversity analyses at the amino acid level for participants with a baseline sequence and ≥1 follow-up timepoint (SNG001, n = 75 participants, 185 timepoints; Placebo, n = 61 participants, 150 timepoints)
- Average pairwise distance (APD) and emergent polymorphisms were evaluated.
- Statistical comparisons between active and placebo groups using Mann-Whitney and Fisher Exact tests without adjustment for multiple comparisons.
  - Stratifications by variant and timing of study entry (<5 or ≥5 days from symptom onset).

RESULTS

- There was a trend of lower amino acid diversity in inhaled IFN-β1a recipients (**Figure 1A**).
- This pattern was more prominent in those that entered the study <5 days from symptom onset (**Figure 1B**) than those who enrolled in the study ≥5 days from symptom onset (**Figure 1C**).
- We saw no difference in viral diversity by timing of study entry and variant (**Figure 1D&E**).
- Normalized to SARS-CoV-2 gene length, fewer emergent nonsynonymous mutations occurred in SNG001 recipients, which was significant for three genes (**Figure 2**):
  - ORF1a (SNG001 vs placebo, median 1.51×10^{-4} vs 4.54×10^{-4} mutations per Kb, p=0.04)
  - ORF3a (1.21×10^{-3} vs 2.42×10^{-3}, p=0.03)
  - Spike (5.23×10^{-4} vs 11.77×10^{-4}, p=0.04)
- We did not detect viral signatures of escape in participants who received inhaled interferon treatment. The three emerging polymorphisms with significantly different (all p>0.05) prevalence between arms occurred more frequently in placebo participants (**Figure 3**).

Figure 1: SNG001-treated arm has lower amino acid average pairwise distance

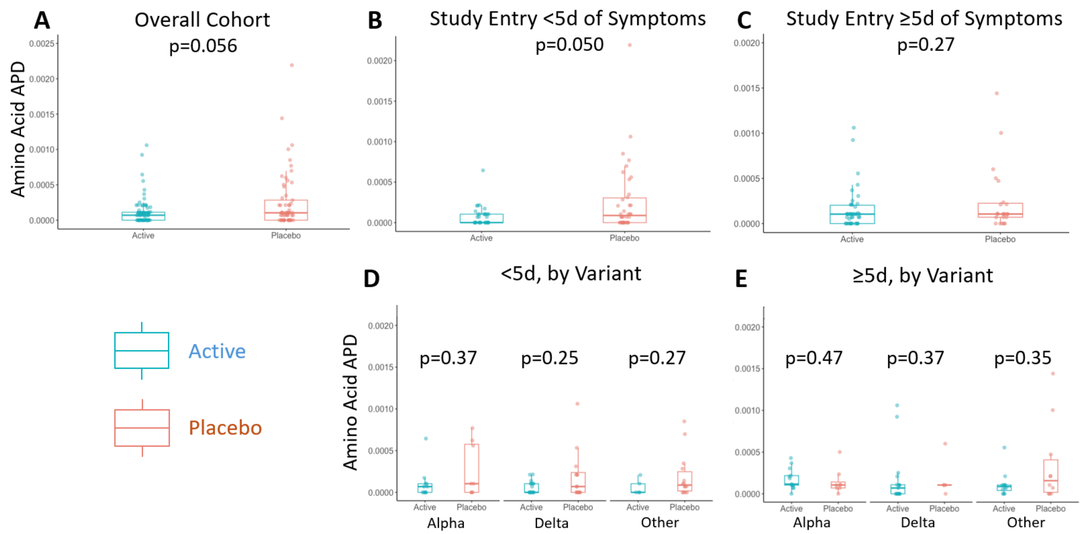


Figure 2: SNG001-treated arm has lower emerging mutations

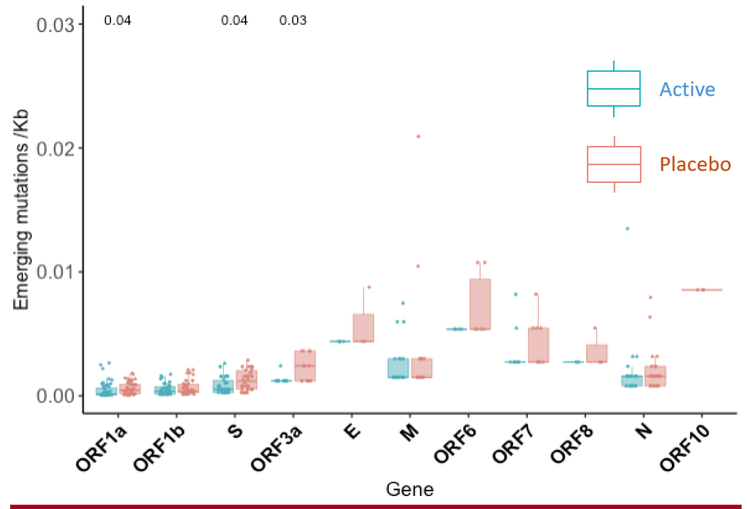
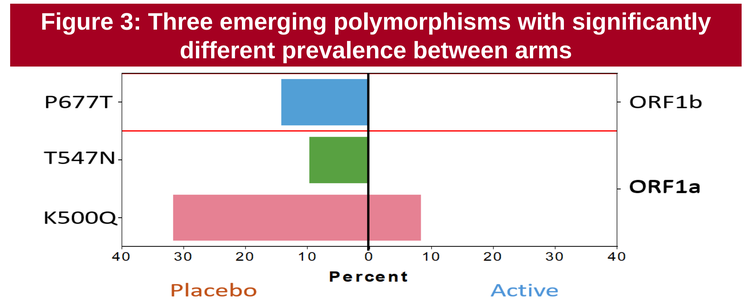


Figure 3: Three emerging polymorphisms with significantly different prevalence between arms



CONCLUSIONS

- Treatment with inhaled SNG001 was associated with lower viral sequence diversity and less frequent emerging amino acid polymorphisms in SARS-CoV-2 isolated from nasal swabs.
- These *in vivo* data provide an intriguing signal that IFN may prevent viral diversification and escape, despite a lack of accelerated nasal viral RNA decay.
- Our study adds to growing evidence that early interferon activity may play a critical role in antiviral responses during COVID-19

ACKNOWLEDGEMENTS

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