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Pharmacokinetics and Safety Profile of SNS812, a First in Human Fully Modified siRNA Targeting Wide-Spectrum SARS-CoV-2, in Healthy Subjects

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ABSTRACT

Severe acute respiratory syndrome caused by the coronavirus (SARS-CoV-2) in the COVID-19 pandemic has highlighted the need for effective treatments, as rapid viral mutations complicate therapeutic development. SNS812, a fully modified inhaled siRNA that targets the conserved RNA-dependent RNA polymerase (RdRP) gene of SARS-CoV-2, has been shown to possess its suppression ability against wide-spectrum SARS-COV-2 variants preclinically. To evaluate the safety and tolerability of inhaled SNS812 in healthy participants, a randomized, double-blind, placebo-controlled phase 1 trial was conducted. To justify the first-in-human inhalation study, this research was divided into two parts: single ascending doses (0.3, 0.6, and 1.2 mg/kg) and multiple doses (0.6 and 1.2 mg/kg) of daily inhalation for seven consecutive days to assess the safety, tolerability, immunogenicity, and pharmacokinetics of SNS812. Of the 44 participants, 3 in the 0.3 mg/kg single-dose group, 2 in the 1.2 mg/kg multiple ascending doses group, and 1 in the placebo group reported treatment-emergent adverse events (TEAEs). No serious adverse events (SAEs), treatment-related adverse events (TRAEs), or TEAEs caused discontinuation or deaths were observed. PK showed rapid absorption of SNS812, with peak concentrations (median T_{max}) reached at 1.5–2 h, and an elimination half-life ($t_{1/2}$) between 4.96 and 7.08 h. No antidrug antibodies (ADAs) were detected in either group. The results demonstrated that the first-in-human, fully modified with wide-spectrum anti-SARS-COV2 siRNA by inhalation following a single dose and multiple doses was safe and well tolerated in healthy participants.

Trial Registration: NCT05677893

1 | Introduction

The novel coronavirus (SARS-CoV-2) has posed a severe threat to human life and disrupted social progress, the World Health Organization (WHO) reporting nearly 776 million confirmed COVID-19 cases globally and a death toll exceeding 7.06 million [1]. Initially, SARS-CoV-2 vaccines were highly effective in reducing both the incidence and severity of the disease [2–4]. However, the emergence of new variants has reduced the virus's sensitivity to therapeutic neutralizing antibodies, convalescent plasma, and vaccines [5]. These challenges underscore the urgent need for new and effective therapeutic strategies.

Summary

- What is the current knowledge on the topic?
  - Rapidly evolved respiratory RNA viruses, such as SARS-CoV-2, influenza, and RSV, pose challenges for existing treatments. RNA interference (RNAi)-based therapeutics offer a precise antiviral approach by silencing essential viral genes. Inhaled siRNA may enhance pulmonary delivery with minimal systemic exposure, while human clinical data on lung-delivered siRNA remain limited.
- What question did this study address?
  - This Phase I trial evaluated the safety, tolerability, and pharmacokinetics (PK) of SNS812, an inhaled siRNA drug targeting SARS-CoV-2's RNA-dependent RNA polymerase (RdRP), in healthy volunteers. The study also assessed anti-drug antibody (ADA) formation.
- What does this study add to our knowledge?
  - SNS812 was well tolerated across all dose levels, with no serious adverse events. PK analysis showed that no ADA formation was detected, supporting low immunogenicity and suitability for repeated dosing.
- How might this change clinical pharmacology or translational science?
  - SNS812 provides informative human data on inhaled siRNA therapy, supporting its potential as a next-generation antiviral for SARS-CoV-2 and other rapidly evolving respiratory viruses. These findings advance pulmonary RNAi drug development and pandemic readiness efforts.

During the recent pandemic caused by SARS-CoV-2, siRNA therapy has emerged as a highly promising approach to combat the virus and its rapidly evolving variants [6]. By targeting a highly conserved region of SARS-CoV-2, siRNA can effectively inhibit a wide spectrum of viral variants and could thus be a one-for-all therapy for the rapidly evolving SARS-CoV-2 by specifically knocking down the coronaviruses' mRNA and protein expressions, disrupting their replication and spread [7, 8]. Among potential siRNA candidates, the RNA-dependent RNA polymerase (RdRp) serves as the most specific target for RNA viruses, exhibiting notable differences between positive-sense and negative-sense RNA viruses [9–11].

SNS812 (also known as MBS-COV C6G25S) is a fully modified siRNA that specifically targets a highly conserved region of the SARS-CoV-2 RdRP gene [11], which has remained unchanged since the emergence of SARS-CoV-1 in 2003 and SARS-CoV-2 in 2019, even with the recent outbreak strain BA2-like_XEC (Figure 1). Preclinical studies have demonstrated its wide-spectrum antiviral activity against SARS-CoV-2 infection [11]. SNS812 is the sodium salt of a chemically synthesized double-stranded oligonucleotide, with a 19-base (19-mer) sense strand and a 21-base (21-mer) antisense strand. Its modifications include 2′-O-methyl, 2′-fluoro, and phosphonothioate (PS) substitution, which enhances its stability and therapeutic efficacy. This design accounts for its effectiveness against 99.8% of current SARS-CoV-2 variants, including the ability to inhibit dominant strains like Alpha, Delta, Gamma, and Epsilon in vitro [11].

It is generally believed that macromolecules, such as siRNA, should be delivered through specialized delivery systems (e.g., Tri-GalNAc or lipid nanoparticles, LNPs) to target specific cells. However, our recent study clearly demonstrated that naked,

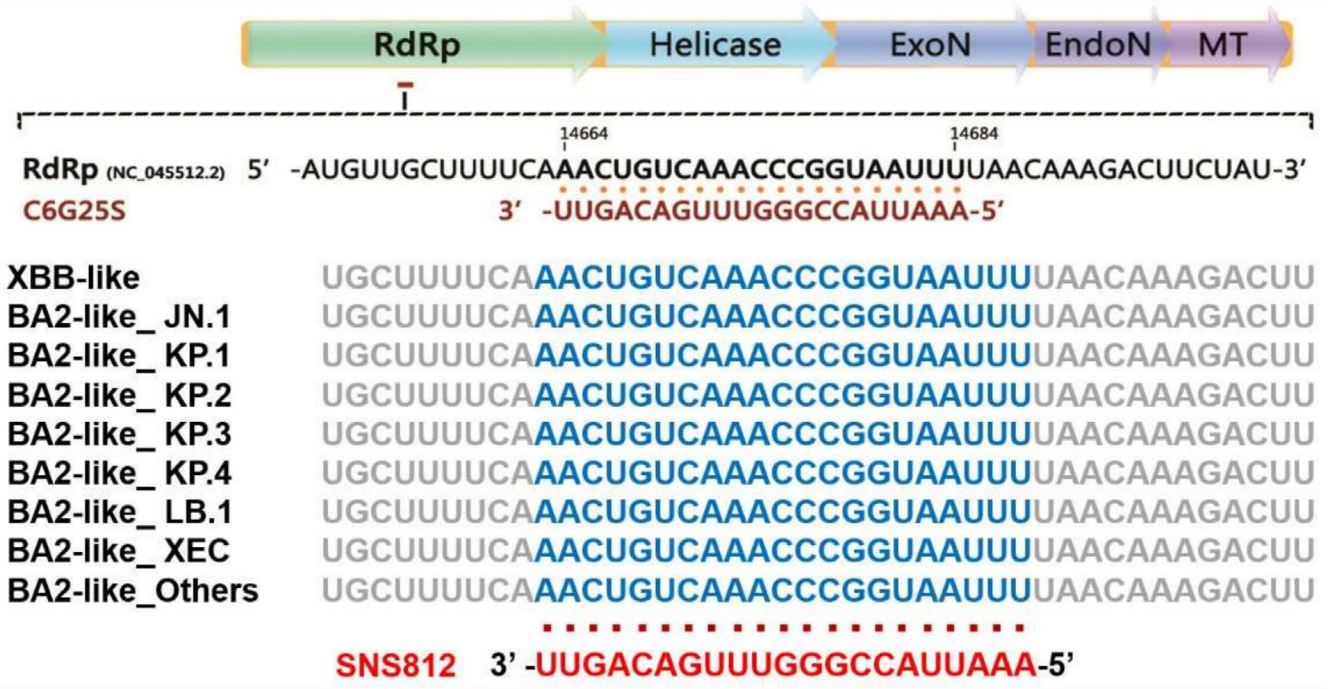

FIGURE 1 | Genetic map of SARS-CoV variations and the target region of SNS812. The sequence of SNS812 targets a highly stable region of the virus RdRP (accession number: NC_045512.2).

fully modified siRNA can be effectively distributed in the lungs via aerosol inhalation, efficiently targeting SARS-CoV-2 variants in infected ACE2 mice [11]. Thus, an excipient- and LNP-free formulation for SNS812 was designed to reduce unwanted side effects. Through a series of preclinical trials, we validated the pharmacological activity and toxicity of SNS812 in both cellular and animal models. This trial aims to assess its safety. pharmacokinetics (PKs), and immunogenicity in healthy adults.

2 | Methods

2.1 | Study Design

This phase I clinical trial was a double-blind, randomized, placebo-controlled study designed to assess the safety, tolerability, and PKs of inhaled single dose-escalation or multiple increasing doses of SNS812 in healthy adults. The trial is conducted by Altasciences Clinical Los Angeles Inc. from November 1, 2022, to March 8, 2023. This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and received approval from the ADVARRA Institutional Review Board (IRB) and was approved on October 20, 2022 for the approved number TMA-P2-872. Additionally, all participants provided written informed consent prior to their enrollment in the study. Participants underwent a screening visit within 28 days prior to enrollment to determine eligibility. A total of 44 healthy participants were recruited and randomly assigned to receive either SNS812 or placebo at a designated dose on Day 1.

The study was divided into two parts. Part A was the single ascending dose (SAD) phase, in which a total of 24 participants were equally allocated to three dose levels (0.3, 0.6, and 1.2 mg/kg) to eight participants per group. The SAD phase employed a sentinel design, with two participants initially enrolled per dose, followed by the remaining six if the dose was well tolerated for 48 h. Dose escalation decisions were made by the Safety Review Committee after thorough safety assessments. Part B, the multiple ascending dose (MAD) phase, commenced after the safety review. In this phase, 20 participants were divided into two dose groups (0.6 mg/kg, 1.2 mg/kg) to 10 participants per group and received treatment for seven consecutive d. Participants were then monitored for 21 days to assess posttreatment safety. A sentinel design was again used, with early participants at each dose level closely monitored before proceeding with further enrollment at that dose. PK evaluation of SNS812 was conducted pre- and post-dosing on Day 1, Day 2, and Day 3 during the SAD phase and from Day 1 to Day 7, as well as on Day 8 and Day 9 during the MAD phase. Anti-drug antibodies (ADA) were assessed 14 and 28 days after the first dose in both the SAD and MAD phases.

2.2 | Participants

Eligible participants that met the inclusion and exclusion criteria were defined as healthy adults. The criteria were briefed as aged 18–55 with a body mass index (BMI) between 18.0 and 32.0 kg/m^2, normal spirometry values, not having smoked for at least 3 months, normal laboratory results including hematology, biochemistry, coagulation indices, and urinalysis, normal 12-lead ECG and chest X-ray at screening, and no chronic diseases or active pulmonary or sinus conditions. Eligible participants should be free of severe systemic or chronic disease. Participants with a history or presence of active lung disease (i.e., asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, hemoptysis, and bronchiectasis), acute sinusitis or a history of chronic sinusitis, nasopharyngeal abnormality, or an upper respiratory infection within the 3 months prior to the first dose would be excluded. In addition, the COVID-19 antigen rapid test at screening and the qRT-PCR test on Day 1 should have been negative.

2.3 | Randomization

Randomization was conducted using a web-based system. Participants who provided written informed consent and met all inclusion criteria without any exclusion criteria were randomly assigned to one of the treatment groups. The total eligible participants were divided into Part A (SAD phase) with three randomized dosing groups (0.3, 0.6, 1.2 mg/kg of SNS812). Eight participants in each group were randomized in a 6:2 ratio for SNS812 versus placebo, starting with the lowest dose. After reviewing the safety results from Part A, Part B of the study, known as the MAD phase, was initiated. In Part B (MAD phase), a total of 20 participants were equally divided into two dosing groups (0.6 and 1.2 mg/kg of SNS812), with randomization occurring in an 8:2 ratio (SNS812: placebo).

2.4 | Intervention

SNS812 is provided as a sterile, excipient-free lyophilized powder containing 50 mg of siRNA (manufactured at STA Pharmaceutical) in a single-dose 2-mL vial. Prior to inhalation, it is reconstituted with normal saline at a determined dose level in milligrams per kilogram per day, administered once daily for 1 day in Part A or for seven consecutive days in Part B of the study. The SNS812 powder was substituted with normal saline (0.9% sodium chloride injection) for use as a placebo in both the single- and multiple-dose groups. Both formulations were delivered using a portable nebulizer (vibrating mesh nebulizer Air Pro III). Participants were assigned to receive one of up to three doses of SNS812 (0.3, 0.6, and 1.2 mg/kg), or placebo, with doses administered sequentially in increasing amounts. Regarding the fixed aerosol spray speed of the nebulizer, the dosage for each group was calculated based on the subjects' body weight to determine their inhalation duration. The duration of nebulization varied by dosing group for subjects weighing between 50 and 100 kg. The times were as follows: 12 to 17 min for the 0.3 mg/kg group, 13 to 18 min for the 0.6 mg/kg group, and 21 to 29 min for the 1.2 mg/kg group.

2.5 | Outcomes

The primary outcome measures of this study were the evaluation of the safety and tolerability of SNS812 in single- and multiple-dose regimens among healthy participants. These parameters were evaluated based on the incidence and severity of treatment-emergent adverse events (TEAEs), the rate of withdrawals due to adverse events (AEs), the incidence of treatment-related

adverse events (TRAEs), the occurrence of serious adverse events (SAEs), and any changes in laboratory values, vital signs, and other safety examination parameters from baseline.

2.6 | PK Sample Collection and Analysis

In the SAD cohorts, blood samples were collected pre-dosing and at multiple post-dosing time points: 5 min (±1 min), 15 min (±2 min), 0.5 h (±5 min), 1 h (±5 min), 2 h (±5 min), 4 h (±5 min), 8 h (±10 min), 10 h (±10 min), 24 h (±10 min), and 48 h (±10 min). For the MAD cohorts, blood samples were collected pre-dosing daily from Day 1 to Day 7, with post-dosing samples taken at the same intervals on both Day 1 and Day 7. Plasma concentrations of SNS812 were quantified using a validated stem-loop RT-qPCR method. This method involved reverse transcription of total RNA in human plasma using the SuperScript IV First-Strand Synthesis System (Invitrogen), followed by a stem-loop cDNA primer: 5′-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACA ACT GTC A-3′. Quantitative PCR amplification was conducted using a PowerTrack SYBR Green Master Mix (Applied Biosystems), with the QuantStudio 7 Flex Q-PCR System (Applied Biosystems) utilized for analysis. Forward primer: 5′-AAG CGC CTA AAT TAC CGG GTT-3′; reverse primer: 5′-GTG CAG GGT CCG AGG T-3′.

The assay was validated by Frontage Laboratories Inc., following FDA guidelines. The positive control for this validation method is the SNS812 (Lot number: CT-22E002). All standards, QC samples, and validation samples were tested in triplicate wells. The final raw data is recorded as the Ct value, which is then converted to a base-10 logarithmic concentration (Log10(Conc.)) using a linear regression model. The endpoint quantification is expressed as the SNS812 concentration (ng/mL). The assay demonstrated a sensitivity range from a lower limit of quantitation (LLOQ) of 5.28552×10^{-3} ng/mL to an upper limit of quantitation (ULOQ) of 5.28552×10^{1} ng/mL. This validation includes accuracy and precision (A&P), sensitivity (LLOQ), dilution linearity (DL), selectivity, stability (benchtop/freeze–thaw/stock solution), and long-term stability (LTS).

2.7 | Pharmacokinetic Assessments

Pharmacokinetic (PK) assessments included dose proportionality evaluation using the PK concentration analysis set (PKCS) and PK parameter analysis set (PKPS). The main PK parameters are as follows: C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, AUC_{tau}, AUC_{0-inf}, CL/F, Vd/F, λz, $C_{min,ss}$, $C_{max,ss}$, $C_{avg,ss}$, $t_{1/2,ss}$, and $T_{max,ss}$. PK parameters were analyzed in participants who had no major protocol violations and at least one analyzable PK parameter. The PK parameters will be derived using Phoenix WinNonlin version 8.3.1 or higher (Certara, Princeton, NJ, USA); SAS version 9.4 or higher (SAS Institute, Cary, North Carolina, USA) was used for the PK statistical analysis.

2.8 | Immunogenicity

Blood samples were collected from participants on Day 1 (predose), Day 14, and Day 28 to assess the levels of anti-SNS812 polyclonal antibodies in human serum. Immunogenicity analysis was conducted by Frontage Laboratories Inc., using a validated and standardized enzyme-linked immunosorbent assay (ELISA) method in compliance with US Food and Drug Administration (FDA) guidelines. The detection of anti-SNS812 polyclonal antibodies followed a multi-tiered anti-drug antibody (ADA) testing approach, which included screening, confirmatory, and titration phases. Positive samples were required to meet the coefficient of variation (%CV) acceptance criteria: ≤20.0% for high-positive control (HPC) and low-positive control (LPC), and ≤25.0% for negative control (NC). The primary endpoint of the immunogenicity analysis was to determine the incidence of anti-SNS812 antibodies following SNS812 administration. The immunogenicity set (IS) consisted of all participants who received at least one dose of the study drug and had at least one ADA concentration measurement. Data from the IS were analyzed to evaluate the presence and persistence of anti-SNS812 antibodies over the study period.

2.9 | Statistical Analysis

A total of 44 participants were recruited for the study, with 24 in Part A and 20 in Part B. The sample size was determined based on clinical judgment rather than statistical power, as it was deemed sufficient to provide relevant descriptive statistics in line with the study objectives.

The safety analysis set included all participants who received the study drug at least once. Statistical calculations related to safety were performed using SAS (Version 9.4 or above). Frequencies of participants and events were summarized by treatment group, along with all AEs. Categorical variables were presented as percentages. The safety and tolerability of the therapeutic regimens were assessed based on AEs, 12-lead ECGs, vital signs, and clinical and laboratory findings. Verbatim AE terms were coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 26.0, and classified by organ system and preferred terms.

PK concentration analyses were conducted on participants who received at least one dose of the study drug, assessing their plasma concentration. Plasma concentrations were summarized by dose level and planned sampling time. Mean and median concentration–time profiles for both the SAD and MAD phases were plotted for each dose level on semilogarithmic scales. The steady state of SNS812 was evaluated using a mixed model, analyzing trough concentrations (C_{min}) from Day 2 pre-dose to the last administration. PK parameters were quantified using non-compartmental analysis with Phoenix WinNonlin version 8.3.5 software based on the actual sampling times.

3 | Results

This phase I, double-blind randomized study was conducted from November 1, 2022, to March 8, 2023, at a single center in the United States. Out of 197 participants screened, 153 were not eligible for enrollment due to not meeting the inclusion and exclusion criteria. A total of 44 healthy participants (28 males (63.6%) and 16 females (36.4%); mean age [SD], 35.39 [8.24])

were ultimately included and randomized to their assigned dosages, with 24 participants in Part A (SAD phase) and 20 in Part B (MAD phase).

In Part A of the study, with a 4:2 allocation ratio of SNS812: placebo, 18 participants received SNS812, distributed among three dosing groups: 0.3, 0.6, and 1.2 mg/kg, and six participants received a placebo. All but one (4.2%) participant who received 0.3 mg/kg SNS812 withdrew early from the study; the remaining 23 (95.8%) participants completed the study (Figure 2).

In Part B of this study, 16 participants received SNS812, with eight participants assigned to 0.6 mg/kg and the other eight participants to 1.2 mg/kg, while four participants were given a placebo. Two (10.0%) participants withdrew early from the study—one from the 0.6 mg/kg group and the other from the 1.2 mg/kg group—while the remaining 18 (90.0%) participants completed the study (Figure 2).

The mean age (SD) was 34.79 (8.77) years in Part A and 36.10 (7.62) years in Part B. In Part A, 18 male participants (75%) were randomized, compared to 10 males (50%) in Part B. The mean weight (SD) was 77.38 (12.1) kg in Part A and 75.36 (12.68) kg in Part B. The mean BMI was 26.22 (2.72) kg/m^2 in Part A and 26.35 (2.96) kg/m^2 in Part B. None of the participants had a history of smoking. The demographic characteristics across all groups were similar, with the majority of White participants ($n = 18$, 75% in part A; $n = 8$, 40% in part B) (Table 1).

3.1 | Primary Efficacy: Safety

In this trial, no subjects withdrew due to adverse events (AEs). Seven cases of AEs were reported, which were all treatment-emergent adverse events (TEAEs) and listed in Table S1. In the SAD phase, four (16.7%) participants experienced five TEAEs. Of these, three participants were assigned to the 0.3 mg/kg SNS812 group; reported included headaches (2 cases), lightheadedness (1 case), and noninfective bronchitis (1 case), while one participant received a placebo and experienced skin abrasions (1 case). Most TEAEs were observed in participants who received 0.3 mg/kg SNS812 (3 participants). All TEAEs were deemed unrelated to the treatment, with all events classified as Grade 1 in severity. All AEs were resolved without intervention. No SAEs and TRAEs were reported in the SAD phase (Table 2).

In the MAD phase, two participants experienced TEAEs, both of whom received 1.2 mg/kg SNS812. The specific TEAEs identified in Part B were throat irritation (1 case) and rash (1 case). All events were classified as Grade 1 in severity. None of the

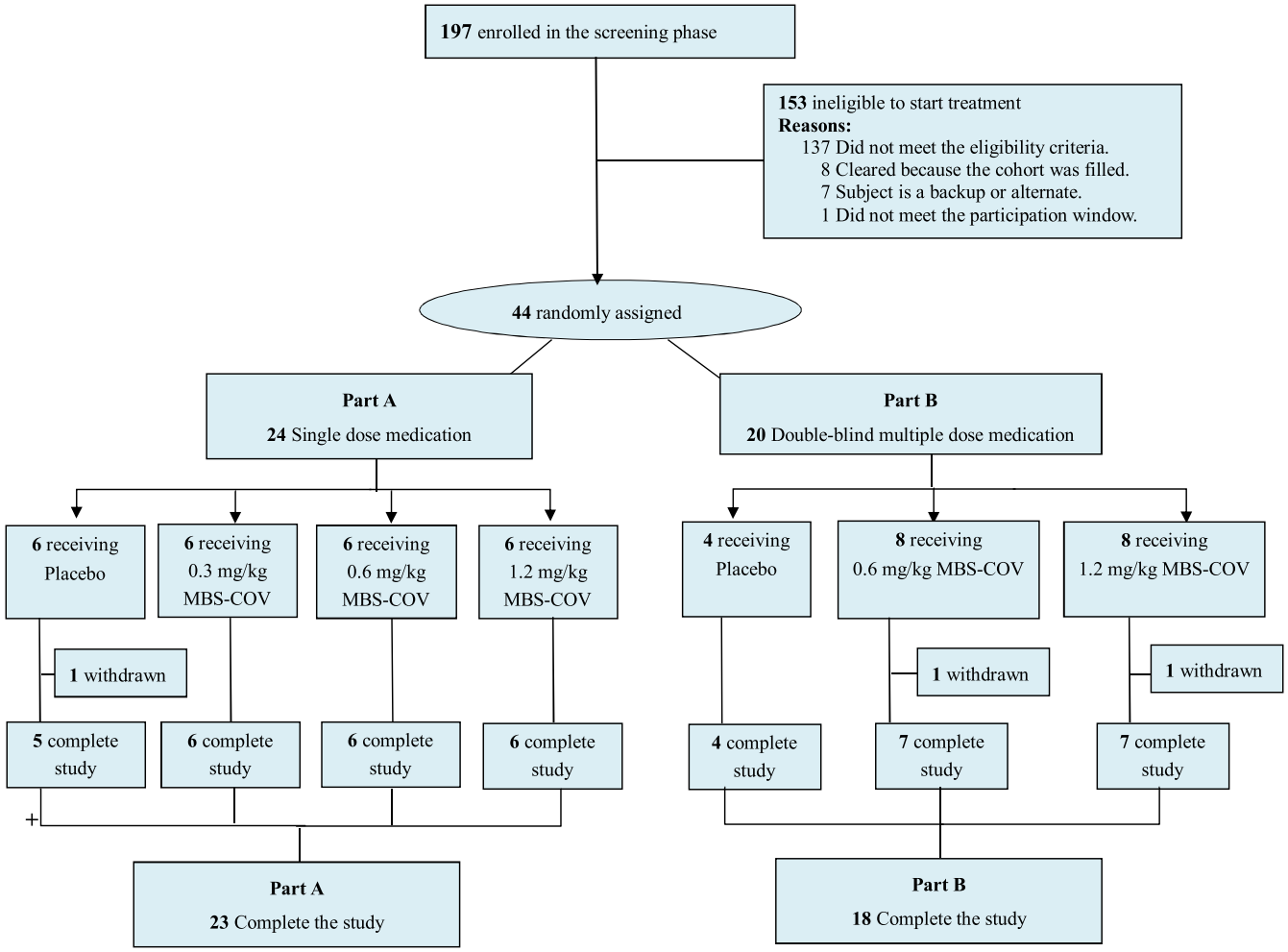

FIGURE 2 | CONSORT diagram of study flow. A total of 197 participants were screened, of which 153 did not meet the eligibility criteria. Ultimately, 44 participants were enrolled and randomized into dosage regimens, with 24 in Part A and 20 in Part B.

TABLE 1 | Baseline characteristics of patients in Parts A and B.

	Part A (SAD)					Part B (MAD)			
Characteristic	**0.3 mg/kg (*N* = 6)**	**0.6 mg/kg (*N* = 6)**	**1.2 mg/kg (*N* = 6)**	**Placebo (*N* = 6)**	**Overall (*N* = 24)**	**0.6 mg/kg (*N* = 8)**	**1.2 mg/kg (*N* = 8)**	**Placebo (*N* = 4)**	**Overall (*N* = 20)**
Age (years)									
Mean (SD)	37.67 (9.09)	38.83 (7.47)	30.83 (6.8)	31.83 (10.5)	34.79 (8.77)	35.13 (5.38)	36.38 (9.61)	37.5 (8.89)	36.1 (7.62)
Gender, *n* (%)									
Male	5 (83.3)	5 (83.3)	2 (33.3)	6 (100.0)	18 (75.0)	6 (75.0)	4 (50.0)	0	10 (50.0)
Female	1 (16.7)	1 (16.7)	4 (66.7)	0	6 (25.0)	2 (25.0)	4 (50.0)	4 (100.0)	10 (50.0)
Ethnicity, *n* (%)									
Hispanic or Latino	1 (16.7)	3 (50.0)	3 (50.0)	1 (16.7)	8 (33.3)	1 (12.5)	2 (25.0)	1 (25.0)	4 (20.0)
Not Hispanic or Latino	5 (83.3)	3 (50.0)	3 (50.0)	5 (83.3)	16 (66.7)	7 (87.5)	6 (75.0)	3 (75.0)	16 (80.0)
Race, *n* (%)									
American Indian or Alaska Native	0	0	0	1 (16.7)[a]	1 (4.2)	0	0	0	0
Asian	0	0	0	0	0	1 (12.5)	0	1 (25.0)	2 (10.0)
Black or African American	2 (33.3)	2 (33.3)	0	2 (33.3)[a]	6 (25.0)	4 (50.0)	2 (25.0)	1 (25.0)	7 (35.0)
White	4 (66.7)	4 (66.7)	6 (100.0)	4 (66.7)[a]	18 (75.0)	2 (25.0)	5 (62.5)	1 (25.0)	8 (40.0)
Other	0	0	0	0	0	1 (12.5)	1 (12.5)	1 (25.0)	3 (15.0)
Height, cm									
Mean (SD)	175.77 (8.85)	169.63 (7.93)	167.6 (9.72)	173 (6.49)	171.5 (8.40)	167.94 (9.31)	174.11 (11.1)	160.1 (9.87)	168.84 (10.99)
Weight, kg									
Mean (SD)	83.17 (16.12)	77.1 (9.74)	71 (9.92)	78.23 (11.44)	77.38 (12.10)	74.61 (10.16)	79.13 (16.2)	69.3 (8.76)	75.36 (12.68)
BMI (kg/m^2)									

(Continues)

TABLE 1 | (Continued)

Characteristic	Part A (SAD)					Part B (MAD)			
	0.3 mg/kg (*N* = 6)	0.6 mg/kg (*N* = 6)	1.2 mg/kg (*N* = 6)	Placebo (*N* = 6)	Overall (*N* = 24)	0.6 mg/kg (*N* = 8)	1.2 mg/kg (*N* = 8)	Placebo (*N* = 4)	Overall (*N* = 20)
Mean (SD)	26.77 (3.5)	26.75 (2.21)	25.22 (1.90)	26.13 (3.35)	26.22 (2.72)	26.49 (3.22)	25.89 (3.40)	27 (1.78)	26.35 (2.96)
Smoking history									
Yes	0	0	0	0	0	0	0	0	0
No	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	24 (100.0)	8 (100.0)	8 (100.0)	4 (100.0)	20 (100.0)

[a]One subject in the placebo group was dual race.

TEAEs were considered related to the treatment, and all resolved without intervention during the treatment period. No SAEs or TRAEs were reported in the MAD phase (Table S1).

There were no TEAEs associated with physical evaluations, laboratory results, vital signs, or ECGs in either the SAD or MAD phases. No TEAEs that led to discontinuation of therapy or deaths (Table 2). Although a decreasing trend in neutrophil count was observed from baseline compared to placebo 4 days post-dosing at the highest dose of SNS812 in the MAD cohort, there was no significant difference between groups (Figure S1). In addition, the abnormal values were not considered clinically significant by the PI. No significant differences were found between the groups in the mean values and changes from baseline for other laboratory tests, ECGs, vital signs, or spirometry assessments.

3.2 | Secondary Efficacy: Pharmacokinetics

The PK profiles of SNS812 were measured and calculated through detecting the full-length SNS812 in plasma by stem-loop RT-qPCR after a single inhalation of 0.3, 0.6, and 1.2 mg/kg within 48 h in the SAD phase (Figure 3A). The minimum time taken to reach the SNS812 peak plasma concentration (T_{max}) at each dosing group is between 1.53 and 2 h, indicating SNS812 was rapidly absorbed following inhalation within 2 h (Figures 3A and Table 3). The geometric peak serum SNS812 concentration (C_{max}) was 7.86 ng/mL in the 0.3 mg/kg dosing group, 19.55 ng/mL in the 0.6 mg/kg dosing group, and 175.53 ng/mL in the 1.2 mg/kg dosing group, demonstrating SNS812 was absorbed in a dose-proportional manner. The geometric mean $AUC_{0\text{-}48}$ of SNS812 (0.3, 0.6 and 1.2 mg/kg) was 56.66, 152.96, and 1360.63 h·ng/mL, respectively. The geometric mean $AUC_{0\text{–}inf}$ was 56.94, 155.76, and 1374.92 h·ng/mL, respectively. The value of $AUC_{0\text{–}48}$ was comparable to $AUC_{0\text{–}inf}$ in each dosing group, suggesting SNS812's complete elimination time is around 48 h in all dosing groups. The coefficient of variation of the geometric mean (%CV) of C_{max} ranged from 52.96% to 80.92%; %CV of AUC ranged from 63.13% to 78.69%, indicating a great variation of AUC and C_{max}. The apparent $t_{1/2}$ after single-escalating SNS812 administration is 4.78, 6.25, and 6.78 h, respective to the escalating dosages. The mean CL/F of SNS812 was 6.53, 4.38, and 1.04 L/h/kg, respectively. The mean Vd/F of SNS812 was 44.68, 46.27, and 11.64 L/kg, respectively. The exposure of SNS812 increased in a dose-proportional manner in the SAD phase (Table 3).

In the MAD phase, on Day 1 following inhalation of multiple doses (once a day for 7 days) of 0.6 and 1.2 mg/kg, SNS812 was absorbed rapidly with the median T_{max} being 2.0 h (Figure 3B and Table 3). The geometric mean $AUC_{0\text{–}24}$ of SNS812 (0.6 and 1.2 mg/kg) was 336.84 and 1408.69 h·ng/mL, increased with the dose, while the mean $AUC_{0\text{–}inf}$ was 300.56 and 1056.44 h·ng/mL, which were comparable to their $AUC_{0\text{–}24}$. The geometric mean C_{max} of SNS812 was 35.04 and 163.60 ng/mL. SNS812 on Day 1 of the MAD phase was eliminated with the mean $t_{1/2}$ ranging from 4.96 to 5.80 h. The mean CL/F of SNS812 was 2.24 and 1.74 L/h/kg, while the mean Vd/F was 16.04 and 17.61 L/kg. Although The $AUC_{0\text{-}t}$ in the SAD group at 0.6 mg/kg was 1.88 times greater at the same dosage as the MAD group, the $AUC_{0\text{–}t}$ in the

TABLE 2 | Primary outcomes: safety profile.

	Part A					Part B			
	0.3 mg/kg	**0.6 mg/kg**	**1.2mg/kg**	**Placebo**	**Overall**	**0.6 mg/kg**	**1.2 mg/kg**	**Placebo**	**Overall**
	(*N* = 6)	**(*N* = 6)**	**(*N* = 6)**	**(*N* = 6)**	**(*N* = 24)**	**(*N* = 8)**	**(*N* = 8)**	**(*N* = 4)**	**(*N* = 20)**
Any AE, *n* (%)	3 (50.0)	0	0	1 (16.7)	4 (16.7)	0	2 (25.0)	0	2 (10.0)
Any TEAE, *n* (%)	3 (50.0)	0	0	1 (16.7)	4 (16.7)	0	2 (25.0)	0	2 (10.0)
Any TRAE, *n* (%)	0	0	0	0	0	0	0	0	0
Any SAE, *n* (%)	0	0	0	0	0	0	0	0	0
Any AE leading to death, *n* (%)	0	0	0	0	0	0	0	0	0
System organ class									
Any TEAEs	3 (50.0)	0	0	1 (16.7)	4 (16.7)	0	2 (25.0)	0	2 (10.0)
Respiratory, thoracic, and mediastinal disorders	1 (16.7)	0	0	0	1 (4.2)	0	1 (12.5)	0	1 (5.0)
Throat irritation	0	0	0	0	0	0	1 (12.5)	0	1 (5.0)
Noninfective bronchitis	1 (16.7)	0	0	0	1 (4.2)	0	0	0	0
Nervous system disorders	3 (50.0)	0	0	0	3 (12.5)	0	0	0	0
Headache	2 (33.3)	0	0	0	2 (8.3)	0	0	0	0
Dizziness	1 (16.7)	0	0	0	1 (4.2)	0	0	0	0
Dermatological issues	0	0	0	1 (16.7)	1 (4.2)	0	1 (12.5)	0	1 (5.0)
Injury, poisoning, and procedural complications	0	0	0	1 (16.7)	1 (4.2)	0	0	0	0
Skin abrasion	0	0	0	1 (16.7)	1 (4.2)	0	0	0	0
Skin and subcutaneous tissue disorders	0	0	0	0	0	0	1 (12.5)	0	1 (5.0)
Rash	0	0	0	0	0	0	1 (12.5)	0	1 (5.0)
System organ class	Severity								
Any TEAEs	Grade 1	3 (50.0)	0	0	1 (16.7)	0	2 (25.0)	0	2 (10.0)
	Grade 2	0	0	0	0	0	0	0	0
	Grade 3	0	0	0	0	0	0	0	0
	Grade 4	0	0	0	0	0	0	0	0

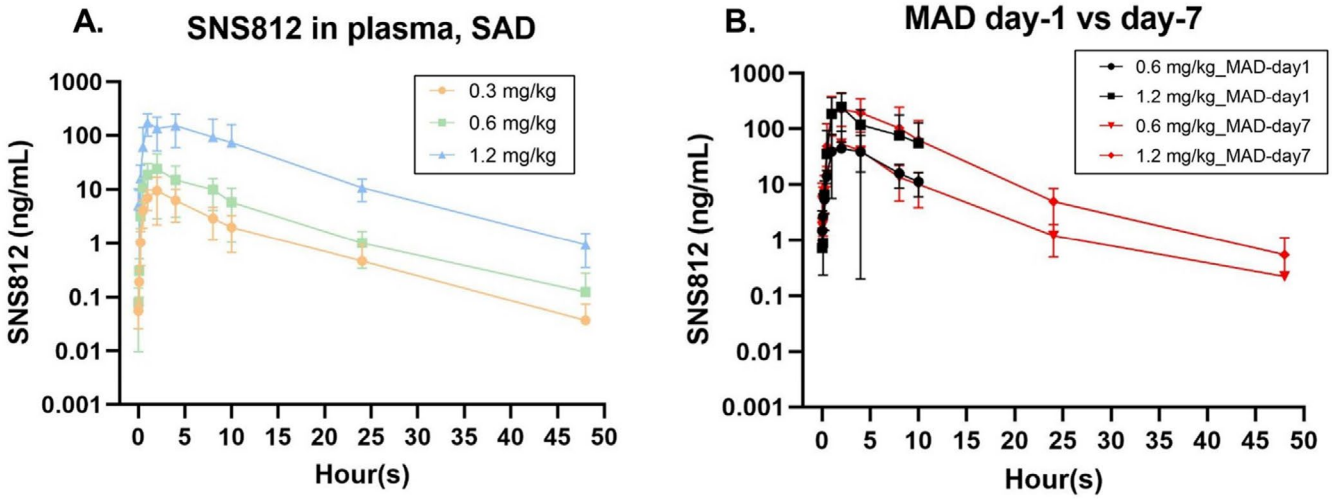

FIGURE 3 | Semilogarithmic scale profiles for SNS812 at each time point in Parts A and B. (A) shows the semilogarithmic scale plasma concentration–time profiles of SNS812 for each dose subgroup in the SAD group. (B) display the semilogarithmic scale plasma concentration–time profiles for each dose group on Day 1 (black line) and Day 7 (red line) in the MAD group.

TABLE 3 | Summary of PK parameters on Day 1 in both groups.

		Cohort (Part A)			Cohort (Part B) Day 1	
PK parameters (units)	**Statistics**	**0.3 mg/kg (*N* = 6)**	**0.6 mg/kg (*N* = 6)**	**1.2 mg/kg (*N* = 6)**	**0.6 mg/kg (*N* = 8)**	**1.2 mg/kg (*N* = 8)**
T_{max} (h)	Median (Min, Max)	2 (1.05, 2.05)	2 (1.00, 2.00)	1.53 (1.00, 8.00)	2 (1.00, 4.00)	2 (1.00, 2.00)
C_{max} (ng/mL)	GeoMean (%CV) Mean ± SD	7.86 (80.92) 9.70 ± 7.25	19.55 (72.76) 24.30 ± 21.35	175.53(52.96) 191.90 ± 78.75	35.04 (73.73) 45.09 ± 45.35	163.60 (198.14) 260.21 ± 190.98
AUC_{0-24h} (h*ng/mL)		—	—	—	288.65 (59.79) 336.84 ± 236.99	985.10 (132.50) 1408.69 ± 1093.41
AUC_{0-48} (h*ng/mL)		56.66 (78.55) 67.06 ± 35.47	152.96 (66.28) 179.138 ± 118.58	1360.63 (78.06) 1668.67 ± 1214.19	—	—
AUC_{0-inf} (h*ng/mL)		56.94 (78.69) 67.40 ± 35.62	155.76 (63.13) 180.67 ± 117.36	1374.92 (77.08) 1679.43 ± 1211.09	300.56 (59.09) 348.69 ± 236.89	1056.43 (121.94) 1469.72 ± 1126.46
$t_{1/2}$ (h)		4.78 (36.51) 5.04 ± 1.75	6.25 (31.35) 6.50 ± 2.10	6.78 (32.93) 7.08 ± 2.23	4.90 (18.45) 4.96 ± 0.83	5.37 (41.58) 5.80 ± 2.74
CL/F (L/h/kg)		5.26 (78.69) 6.53 ± 4.84	3.85 (63.13) 4.38 ± 2.30	0.87 (77.08) 1.039 ± 0.614	2.00 (59.09) 2.237 ± 1.04	1.14 (121.94) 1.74 ± 1.89
V_d/F (L/kg)		36.36 (81.61) 44.68 ± 31.34	34.71 (104.98) 46.27 ± 39.48	8.54 (120.2) 11.64 ± 8.34	14.01 (69.59) 16.04 ± 6.79	8.80 (192.27) 17.61 ± 22.27
MRT (h)		7.01 (44.66) 7.53 ± 3.00	8.81 (22.42) 8.99 ± 1.97	9.26 (20.46) 9.42 ± 1.99	7.5 (18.6) 7.60 ± 1.24	7.32 (51.97) 8.19 ± 4.55

Note: Estimated steady-state PK parameters for multiple-dose administration included $AUC_{0-\tau}$.
Abbreviations: AUC, area under the plasma drug concentration–time curve; $AUC_{0-\infty}$, AUC from time 0 to infinity; AUC_{0-t}, AUC from time 0 to the last quantifiable concentration T_{last}; CL/F, apparent plasma clearance; C_{max}, plasma drug concentration; MRT, mean resident time; $t_{1/2}$, apparent terminal elimination half-life; T_{max}, time to maximum observed plasma drug concentration; V_d/F, apparent volume of distribution.

1.2 mg group was similar, suggesting the difference was due to the large %CV (Table 3). In addition, the kinetic curve between SAD and MAD at the same dosage was quite similar (Figure S2).

The PK profile of 7-day multiple doses of 0.6 and 1.2 mg/kg in the MAD group also indicated SNS812 was absorbed rapidly, with the median $T_{max,ss}$ being 2.0 h (Figure 2B and Table 4). The exposure of SNS812 increased with the dose increased from 0.6 to 1.2 mg/kg, with the geometric mean AUC_{tau} being 262.19 and 1073.57 h·ng/mL, respectively. The geometric mean $C_{max,ss}$ of SNS812 was 37.67 and 170.60 ng/mL. The %CV of $C_{max,ss}$ was 105.37% and 185.56%; %CV of AUC_{tau} was 79.29% and 152.73%, indicating a large variation of AUC and C_{max} (Table 4). SNS812 was eliminated with the mean $t_{1/2,ss}$ ranging from 6.17 to 6.21 h,

TABLE 4 | Summary of PK parameters on Day 7 in the MAD Group.

PK parameters (units)	Statistics	MAD Day 7	
		0.6 mg/kg ($N=7$)	1.2 mg/kg ($N=7$)
$T_{max,ss}$ (h)	Median (min, max)	2 (1.00, 2.03)	2 (1.00, 4.00)
$C_{max,ss}$ (μg/mL)	GeoMean (%CV)	37.67 (105.37)	170.60 (185.56)
	Mean ± SD	54.30 ± 55.47	282.75 ± 232.87
$C_{min,ss}$(μg/mL)		1.14 (41.05)	3.22 (135.59)
		1.22 ± 0.51	4.53 ± 3.48
C_{avg}(μg/mL)		10.92 (79.29)	44.73 (152.73)
		13.875 ± 12.03	70.60 ± 65.32
AUC_{tau} (h*μg/mL)		262.19 (79.29)	1073.57 (152.73)
		333.01 ± 288.66	1694.38 ± 1567.65
$t_{1/2,ss}$ (h)		6.02 (26.77)	5.91 (31.74)
		6.21 ± 1.83	6.17 ± 1.95
CL_{ss}/F (L/h)		2.29 (79.29)	1.12 (152.73)
		2.72 ± 1.52	1.82 ± 1.8
V_{ss}/F(L)		19.86 (115.89)	9.53 (224.57)
		27.47 ± 24.01	18.71 ± 19.79
AR_{AUC}		1.05 (67.55)	1.04 (95.62)
		1.242 ± 0.84	1.26 ± 0.6
AR_{Cmax}		0.89 (49.58)	1.09 (63.12)
		0.97 ± 0.46	1.22 ± 0.50
DF (%)		330.42 (39.31)	366.28 (39.9)
		351.22 ± 131.40	387.04 ± 118.09

Abbreviations: AR_{AUC}, Accuracy rate of AUC; AR_{Cmax}, Accuracy rate of Cmax; AUC_{tau}, Area under the plasma concentration-time curve over a dosing interval; C_{avg}, Average concentration over a dosing interval at steady state; CL_{ss}/F, Apparent clearance at steady state; $C_{max,ss}$, Maximum observed plasma concentration at steady state; $C_{min,ss}$, Minimum observed plasma concentration at steady state; DF, Cmin to Cmax fluctuation between dose time and Tau; $t_{1/2,ss}$, Elimination half-life at steady state; $T_{max,ss}$, Time to maximum plasma concentration at steady state; V_{ss}/F, Apparent volume of distribution at steady state.

and the mean CL_{ss}/F was 2.72 and 1.83 L/h/kg. The mean accumulation ratio (AR) of C_{max} was 0.97 and 1.22, and the mean AR of AUC was 1.24 and 1.26, indicating no obvious accumulation of SNS812 after multiple doses (Table 4).

Anti-SNS812 antibodies were detected by using the ELISA assay, and all of the blood samples were negative at screening, Day 14, and Day 28 in all dosing groups, indicating no potential immunogenicity after administering single and multiple doses (once daily for 7 days) of SNS812 inhalation (Table S2).

4 | Discussion

This phase I, randomized, double-blind study aimed to assess the safety, tolerability, and PK of inhaled SNS812 in healthy adults through single or daily doses over a week. No TRAEs were observed after a single inhalation of SNS812 (0.3–1.2 mg/kg) or during 7 days of SNS812 inhalation (0.6–1.2 mg/kg). There were no deaths or serious TEAEs reported in either phase of the study, and all TEAEs were mild (Grade 1) in severity. No participants discontinued treatment due to TEAEs, which resolved by the end of the study. The results suggest that inhalation of SNS812, a modified double-stranded siRNA, is safe and well-tolerated within the dosing regimen.

SNS812 was rapidly absorbed after inhalation, with peak concentrations observed between 1.5 and 2 h (median T_{max}). The exposure to SNS812 increased with the dose, and the mean elimination half-life ($t_{1/2}$) ranged from 4.96 to 7.08 h. With increased doses in both the SAD and MAD phases, the SNS812 exposure became more than dose-proportional, especially in the 1.2 mg/kg dosing group, indicating potential overdosing in lung exposure.

Clinical trials have shown the feasibility and safety of delivering unmodified naked siRNA via the respiratory route to combat respiratory syncytial virus and prevent bronchiolitis obliterans syndrome [12, 13]. Our results further confirm that inhaled SNS812 has an adequate tolerability profile and is effectively delivered to the lungs while being well tolerated. This suggests that using a naked siRNA agent for targeting thoracic viral infections via inhalation could be an ideal approach. Future studies should further investigate the efficacy of SNS812 against SARS-CoV-2 mutations to help alleviate acute respiratory syndrome. We recognize the limitations of this study. First, the small sample size led to a large standard deviation in PK values. Second, the study did not include participants from all ethnic backgrounds, which may have introduced bias. Third, there was insufficient safety data on patients with co-existing conditions that could have influenced the safety and efficacy of the agent in this cohort.

Author Contributions

C.-W. L. wrote the manuscript. P.-C.Y., Y.-C.C., and C.-F.Y. designed the study. H.-J.H. and J.F.Y. performed the research. Y.-F.C. and Y.-L.C. analyzed the data.

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Conflicts of Interest

Yi-Chung Chang, Yi-Fen Chen, Hui-Ju Ho, Jen Fu Yang, and Ching-Wen Lin were employed by Onenessbio Ltd.; Chi-Fan Yang and Yuan-Lin Chou were employed by Microbio (Shanghai) Biotech Company. Pan-Chyr Yang declares no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.