A randomized, double-blind, Phase 1, single- and multiple-dose placebo-controlled study of the safety and pharmacokinetics of IN-006, an inhaled antibody treatment for COVID-19 in healthy volunteers

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Summary

Background Although COVID-19 is predominantly a respiratory tract infection, current antibody treatments are administered by systemic dosing. We hypothesize that inhaled delivery of a monoclonal antibody may be a more effective and convenient route. We investigated the safety, tolerability, and pharmacokinetics of IN-006, a reformulation of regdanvimab for nebulized delivery by a handheld nebulizer.

Methods A Phase 1 study was conducted in healthy volunteers aged 18–55 a Phase 1 unit in Melbourne, Australia (ACTRN12621001235897). Study staff and participants were blinded to treatment assignment, except for pharmacy staff preparing the study drug. The ratio of active:placebo randomization to each cohort was set at 3:1. The primary outcomes were safety and tolerability. Exploratory outcomes were pharmacokinetics of IN-006 in nasal fluid and serum.

Findings Twenty-three participants were enrolled and randomized across two single dose and one multiple dose cohorts (30 mg or 90 mg single nebulized dose, or seven daily 90 mg doses). There were no serious adverse events. All enrolled participants completed the study without treatment interruption or discontinuation. All treatment-emergent adverse events were transient, non-dose dependent, and graded mild to moderate in severity. Nebulization was well-tolerated and completed in an average of 6 min. Geometric mean nasal fluid concentrations of IN-006 in the multiple dose cohort were 739.8 μg/mL at 30 min after dosing and 1.2 μg/mL at 22 h. Geometric mean serum levels in the multiple dose cohort peaked at 0.51 μg/mL 3 days after the final dose.

Interpretation IN-006 was well-tolerated and achieved concentrations in the respiratory tract orders of magnitude above the IC_{50} range typical of antiviral mAbs. These data support further development of nebulized delivery of antiviral mAbs for respiratory infectious disease.

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Keywords: SARS-CoV-2; Clinical trial; Phase I; Antibodies; Monoclonal; Respiratory system

Introduction

SARS-CoV-2,[1–3] like many viruses that cause acute respiratory infections (ARIs), infects cells almost exclusively via the apical (luminal) side of the airway epithelium, and also primarily buds from infected cells via the apical surface.[4–6] Progeny virus must then travel

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Research in context

Evidence before this study

Prior experiences with systemically administered mAbs for acute respiratory tract infections have often suffered from limited efficacy, due in part to the slow and limited pulmonary distribution of systemically administered mAbs. Few studies have attempted nebulization of mAbs directly into the respiratory tract, likely due to the longstanding dogma that mAbs cannot be stably nebulized.

Added value of this study

Our study investigated the safety, tolerability, and upper respiratory tract and serum pharmacokinetics of IN-006, a reformulation of regdanvimab given by inhalation with a handheld nebulizer. We hypothesized that once daily inhaled delivery of mAbs could sustain high mAb concentrations at the site of infection. The study showed that inhaled IN-006 was very well-tolerated and achieved concentrations in the upper respiratory tract that were orders of magnitude above those previously reported after systemic administration of mAbs.

Implications of all the available evidence

Inhaled delivery of potent antiviral mAbs could potentially offer a more effective and convenient treatment option for COVID-19 and other acute respiratory infections. Achieving high concentrations of mAbs in the respiratory tract immediately after dosing may broaden the time window for initiation of treatment, and likely substantially reduce the total dose required per patient. Future research will focus on evaluating the efficacy of inhaled mAbs in treating patients with acute infections and preventing transmission.

through airway mucus to reach uninfected epithelial cells as the infection spreads from the upper respiratory tract (URT) to the lower respiratory tract (LRT) and the deep lung.[7–9] Neutralizing monoclonal antibodies (mAbs) must therefore reach a high enough concentration in the airway lumen to effectively neutralize the virus and halt the infection.

mAbs distribute very inefficiently from the blood into the respiratory tract fluids, resulting in mAb concentrations in the airway fluids that are orders of magnitude lower than those in the serum, following intravenous (IV) or intramuscular (IM) administration.[10–12] Despite these limitations, IV-administered mAbs against SARS-CoV-2 can be effective in treating infected individuals at high risk of severe COVID-19 when they are given early in the course of the infection,[8,9,13] implying that sufficient amounts of mAb can distribute into the airway lumen. Nevertheless, high doses of mAb are generally required if given IV, reducing the number of treatment courses available from a given supply of drug. Delayed distribution into the lung also limits the treatment window for preventing severe COVID-19.[12]

Nebulization has been used to conveniently deliver protein therapeutics (e.g., Pulmozyme) directly to the lungs. Importantly, direct inhaled delivery can achieve far higher concentrations of drugs in the lungs than can be achieved by IV or IM administration, within minutes. Since the pattern of deposition of nebulized drugs along the respiratory tract is largely determined by the aerosol particle size,[14] it is possible to use a nebulizer that generates a broad aerosol size distribution to deliver drug throughout the entire respiratory tract, from the nasal turbinates in the URT, to conducting airways in the LRT, to the deep lungs. Thus, nebulized delivery is likely the fastest method to achieve inhibitory concentrations of mAb in the airway fluids along the entire respiratory tract. Nebulization also enables convenient self-dosing at home, reducing the burden on patients and on healthcare infrastructure.

We developed IN-006, a reformulation of regdanvimab, for nebulized delivery as an inhaled treatment for COVID-19. Regdanvimab, an IV-dosed human IgG_1 mAb directed against the SARS-COV-2 spike protein receptor binding domain (RBD), was approved in the European Union for adults with COVID-19 who did not require supplemental oxygen and who were at increased risk of progression to severe COVID-19. We previously reported the results from preclinical activities that supported a first-in-human study of inhaled IN-006, including muco-trapping of SARS-CoV-2 virions, GLP nebulization stability studies, and excellent tolerability in GLP inhaled toxicology and GLP tissue cross reactivity experiments.[15] The current report describes a Phase 1 study designed to assess the safety, tolerability, and nasal fluid and serum pharmacokinetics of nebulized IN-006 in healthy adults.

Methods

Clinical study design and participants

This double-blind, placebo-controlled, first-in-human, ascending-dose pharmacokinetic and safety study was conducted in a Phase 1 unit in Melbourne, Australia. This study was prospectively registered in the Australian New Zealand Clinical Trials Registry (ACTRN12621001235897). Eligible participants were enrolled sequentially into three cohorts: a single low dose cohort (30 mg), a single high-dose cohort (90 mg), and a multiple high-dose cohort (seven daily 90 mg doses). For each single dose cohort, a sentinel pair (with one active and one placebo recipient) was initially dosed, followed by a two-day safety monitoring period prior to the dosing of the remainder of the cohort. Advancing to subsequent cohorts was done after review of a summary of all safety parameters available over seven

days after final dosing of the preceding cohort by a Dose Escalation Committee. The DEC was composed of the study principal investigator, the sponsor medical monitor, and a third physician independent of the study (see Acknowledgements). The data in the safety summary was prepared and presented blinded to participant assignment by the data team at the study site. Fig. 1 provides a diagram of the process flow, study structure, and times of pharmacokinetic evaluations.

Ethics

The study was carried out according to the International Council for Harmonisation Good Clinical Practice guidelines and Declaration of Helsinki, and conducted in compliance with local regulatory requirements and was approved by The Alfred Hospital Office of Ethics and Research Governance, Melbourne, VIC, Australia (IRB Protocol: IRB00002274). Informed consent was obtained in advance of all study-related procedures.

Inclusion and exclusion criteria

Eligibility criteria required that participants be adults 18–55 years of age with a body-mass index of 18–32 kg/m^2 who were in good health as judged by medical history, physical exam, clinical chemistry and hematology assessments, electrocardiogram, forced expiratory volume in 1 s (FEV_1) ≥90% predicted, and negative serology for HBsAg, HCV, and HIV antibodies. Participants were required to be non- or light smokers. The FEV_1 threshold was changed to ≥80% predicted after enrolling the first 7 participants.

Participants were excluded for known or suspected symptomatic viral infection or signs of active pulmonary infection or pulmonary inflammatory conditions within 14 days of dosing initiation, a history of airway hyperresponsiveness, angioedema, anaphylaxis, or a positive alcohol breathalyzer test and/or urine drug screen for substances of abuse.

During recruitment of the 7 participants comprising the first single dose cohort, participants who had received a COVID-19 vaccine were excluded. However, due to rapidly increasing local vaccine availability and uptake, this criterion was modified to exclude only those vaccinated within two weeks of initial dosing, or those with plans to be vaccinated within two weeks after completion of dosing.

Study endpoints

The primary endpoints for both the SAD and MD cohorts were the incidence and severity of AEs. This was assessed by monitoring treatment-emergent adverse events, pre- and post-dose vital signs, ECG, FEV_1, SpO_2, hematology and chemistry safety blood tests, and physical examinations. Follow-up continued for 28 days, with assessments on the days indicated in Fig. 1B. For the SAD and MD Cohorts, exploratory endpoints included IN-006 concentration in nasal swabs and serum. During the study, in all cohorts, we also evaluated for potential harms including nervous system effects (e.g., dizziness, dysgeusia, headache, presyncope), respiratory, thoracic, or mediastinal disorders (e.g., cough, oropharyngeal pain, throat irritation), musculoskeletal disorders, complications associated

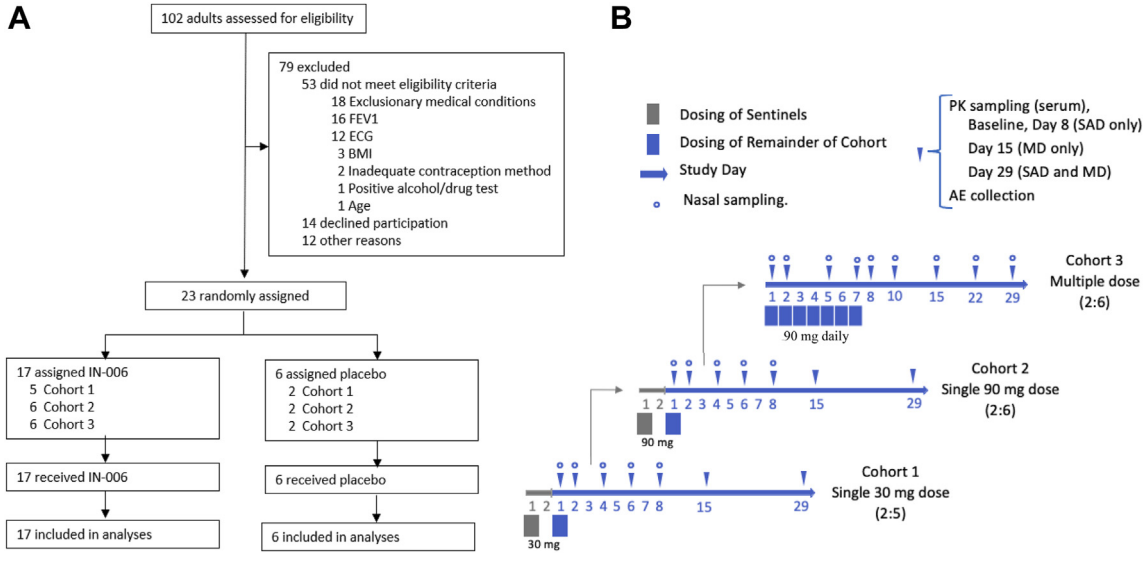

Fig. 1: INH-006-01 clinical trial **(A)** process flow, and **(B)** study structure and times of sample collection for pharmacokinetic evaluations. In panel B, the number of placebo vs. IN-006-treated subjects are shown in parentheses, for example (2:5) in Cohort 1 representing 2 placebo, 5 IN-006 subjects. Together, these figures demonstrate the recruitment of subjects, random assignment, and the timepoints at which samples were collected for analysis.

with the device, and changes in forced expiratory volume or transaminase measurements.

Randomization and masking

A randomization schedule was prepared using validated software (SAS, PROC PLAN) by an unblinded statistical team members who had no responsibility for monitoring and data management of this study, with provisions for each sentinel pair to include one active and one saline placebo assignment, and for the overall ratio of active to placebo assignment of each cohort to be 3:1. The randomization code was held by unblinded pharmacy staff who prepared the doses in matching syringes with identical appearances for loading into the nebulizer by clinical staff. The randomization sequence was maintained under controlled access, with a copy of the randomization sequence provided to an unblinded site pharmacy staff member responsible for randomizing the subject and dispensation of study drug. Other study staff, participants, and those assessing outcomes were blinded to assignment.

Interventions/Clinical procedures

IN-006 drug substance was produced under Good Manufacturing Practices (GMP) and supplied as a liquid formulation in glass vials from the manufacturer, Celltrion, Inc. IN-006 was provided in a syringe as a liquid formulation to be loaded into the InnoSpire Go vibrating mesh nebulizer (Koninklijke Philips N.V., cleared under 510 K K170853). Placebo participants received an identical syringe containing saline instead of IN-006, which was also loaded into and administered via the InnoSpire Go Nebulizer.

Participants were instructed to breathe in slowly through the nebulizer mouthpiece and to breathe out through their nose. Nasal fluid was obtained by rotating a flocked swab (Copans Cat. # 56380CS01) for 10–15 s at mid-turbinate depth (4–5 cm). Sampling alternated between right and left nostrils during sequential sample collection timepoints. The amount of nasal fluid sample collected by each individual swab was determined by weighing the sample-containing swab and sample tube before and after it was incubated in buffer for extraction, rinsed, and oven dried. Sampling times for nasal fluid and serum are shown in Fig. 1B. Vital signs and FEV_1 were measured before nebulization and 15 and 30 min after completion of nebulization.

Measurement of IN-006 concentration in human serum and nasal fluid, and PK modeling

ELISAs to measure IN-006 in biological samples were based on anti-idiotypic antibodies that bind the specific hypervariable region on the Fab domain of IN-006, allowing sensitive differentiation of IN-006 from endogenous anti-RBD antibodies in vaccinated or previously infected subjects. Please refer to the online supplement for further details on sample extraction, ELISA design, and PK modeling.

Statistical analysis

Sample size was chosen according to conventions for Phase 1, first-in-human studies. Formal sample size and power calculations were not performed, given that this was a Phase 1 study and no statistical comparisons were made across outcomes for the different treatment groups beyond simple descriptive summary data. Continuous variables were summarized using descriptive statistics including number of non-missing observations, mean, SD, median, minimum, and maximum values. Categorical variables were summarized with frequency counts and percentages. Placebo recipients in different cohorts were pooled. The safety analysis included all randomized participants who received any dose of study drug. The pharmacokinetic population included all participants who received any dose of IN-006. No inferential statistical tests were conducted. Serum PK parameters of IN-006 were determined using Phoenix WinNonlin version 8.3.

Role of the funding source

U.S. Army Medical Research & Development Command provided funding but was not involved in the design or execution of this project, nor in the analysis of data or reporting of study results. Celltrion Inc. provided regdanvimab drug material and contributed to study design and manuscript review but was not involved in clinical data analysis or safety assessments.

Results

From among 102 adults who were screened, 23 participants were sequentially assigned to one of three cohorts. The first participant was randomized on September 22, 2021, and the last participant visit was on December 29, 2021. Of these participants, 17 were randomly assigned to receive IN-006, and 6 were randomly assigned to receive placebo. All 23 participants received their assigned treatment as intended and completed the final study visit on Study Day 29. The study was completed on December 29, 2021. Participant flow is diagrammed in Fig. 1A, and participant demographics are listed in Table 1.

Safety and tolerability

Treatment emergent adverse events (TEAEs) are listed in Table 2. Nebulization of IN-006 was well-tolerated and completed in an average of 6 min for the 90 mg dose (range 4–9 min). Eight (53.3%) of the 15 participants included in the single ascending dose cohorts experienced at least 1 TEAE (6 of 11 receiving IN-006, 2 of 4 receiving placebo). Among the 11 participants receiving IN-006, the most frequently reported TEAEs in the SAD cohorts were headache (2/11; 18.2%) and

	SAD 30 mg		SAD 90 mg		MD 90 mg		Pooled placebo	Overall total
	IN-006 (n = 5)	Placebo (n = 2)	IN-006 (n = 6)	Placebo (n = 2)	IN-006 (n = 6)	Placebo (n = 2)	(n = 6)	(n = 23)
Sex								
Female	1 (20%)	2	3 (50%)	0	2 (33.3%)	1	3 (50%)	9 (39.1%)
Male	4 (80%)	0	3 (50%)	2	4 (66.7%)	1	3 (50%)	14 (60.9%)
Age, years								
Mean ± Standard deviation	30.0 ± 5.7		33.0 ± 11.3		24.7 ± 5.4		35.7 ± 10.5	30.9 ± 9.2
Range	21–37	29, 30	20–50	28, 52	18–31	29,46	28–52	18–52
Race								
White	3 (60%)	2	4 (66.7%)	2	4 (66.7%)	1	5 (83.3%)	16 (69.6%)
Black or African American	0	0	0	0	0	0	0	0
Asian	2 (40%)	0	2 (33.3%)	0	1 (16.7%)	1	1 (16.7%)	6 (26.1%)
American Indian or Alaska Native	0	0	0	0	1 (16.7%)	0	0	1 (4.3%)
Native Hawaiian or other Pacific Islander	0	0	0	0	0	0	0	0
Australian Aboriginal or Torres Strait Islander	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0
Ethnic origin								
Not Hispanic or Latino	5 (100%)	1	6 (100%)	2	5 (83.3%)	2	5 (83.3%)	21 (91.3%)
Hispanic or Latino	0	1	0	0	1 (16.7%)	0	1 (16.7%)	2 (8.7%)
Weight (kg)								
Mean ± Standard deviation	83.7 ± 24.4		68.7 ± 17.0		68.5 ± 16.7		85.2 ± 13.2	76.2 ± 18.5
Range	53.9–111	70.3, 78.8	53–99.8	97.5, 101.8	50.8–87.6	72.7, 90	70.3–101.8	50.8–111

Table 1: **Demographics of enrolled participants.**

oropharyngeal pain (2/11; 18.2%). All but 1 TEAE were mild. One participant who received IN-006 low dose (30 mg) experienced a moderate event (increased transaminases on Day 29), which was not considered to be related to study drug by the investigator due to the long interval (28 days) between dosing and the observed elevation, and the intervening normal transaminases observed at 7 days after dosing. Moreover, there is extensive experience with the intravenous administration of regdanvimab, and liver toxicity was not observed in prior Phase 1 and Phase 2/3 studies at doses of up to 80 mg/kg, wherein systemic (serum) Cmax was more than 3000 times higher than serum Cmax observed after nebulization in our study. Three (3/15; 20.0%) participants experienced at least 1 TEAE considered related to study drug by the investigator. These events included headache, cough, and oropharyngeal pain. All 3 related TEAEs were mild and resolved. There was no evidence of a dose-related effect (Table 2).

In the multiple dose cohort, no TEAEs were reported in participants receiving placebo. Among the 6 participants receiving IN-006, 4 (66.7%) participants experienced at least 1 TEAE. The most frequently reported TEAE was dizziness (2/6; 33.3%). All but 1 TEAE were mild. One participant receiving IN-006 experienced a moderate event (pain in extremity), which was considered unlikely to be related to study drug by the investigator. Two (33.3%) participants experienced at least 1 TEAE considered related to study drug by the investigator. These drug-related TEAEs were dizziness and decrease in FEV_1: the latter was noted 15 min after nebulization, was not associated with symptoms or abnormal vital signs, resolved within 15 min, and did not recur with subsequent doses. Both events were mild. Comparisons of pre- and post-dosing FEV_1 are described in detail in the Supplementary Data File.

No severe TEAEs, SAEs, or TEAEs leading to discontinuations were reported in either the single dose or multiple dose cohorts. The most frequently reported TEAEs in participants receiving IN-006 across all three cohorts were headache (2/23; 11.8%) and oropharyngeal pain (2/23; 11.8%), both appearing only in the SAD cohorts and not in the multiple dose cohort. There were no unexpected safety signals.

Pharmacokinetics

For single dose cohorts, the geometric mean concentrations of IN-006 per mL of nasal fluid were 146 μg/mL and 459 μg/mL for the 30 mg and 90 mg dose, respectively, measured 3 h after dosing; the difference in these values is consistent with a 3-fold increase in the dose administered (Table 3). In the multiple dose cohort, the repeated dosing provided additional opportunities for more nasal concentration measurements across more time points. The nasal concentrations measured 30 min after dosing on Days 1, 2, and 3 were a geometric mean of 607 μg/mL, which was higher than the geometric mean concentration measured 3 h after

	IN-006 30 mg single dose (n = 5)	IN-006 90 mg single dose (n = 6)	IN-006 90 mg 7 daily doses (n = 6)	Placebo (n = 6)
Participants with any treatment-emergent adverse event (TEAE)	3	3	4	2
Participants with nebulization- related TEAE[a]	1	0	2	0
Number of serious adverse events	0	0	0	0
Number of TEAEs	6	5	6	4
Nervous system disorders	3	2	3	3
Dizziness	0	0	3	0
Dysgeusia	0	1	0	0
Headache	3	0	0	3
Presyncope	0	1	0	0
Respiratory, thoracic and mediastinal disorders	1	2	1	0
Cough	1	0	0	0
Oropharyngeal pain	0	2	0	0
Throat irritation	0	0	1	0
Musculoskeletal and connective tissue disorders	0	0	1	0
Pain in extremity	0	0	1	0
General disorders and administration site conditions	0	1	0	1
Complication associated with device[b]	0	1	0	0
Fatigue	0	0	0	1
Investigations	2	0	1	0
Forced expiratory volume decreased	0	0	1	0
Spirometry abnormal	1	0	0	0
Transaminases increased	1	0	0	0

[a]Occurring within 2-h of completing nebulization; (cough, FEV_1 decrease). [b]Contraceptive intrauterine device.

Table 2: **Summary of safety findings and adverse events.**

dosing (94 μg/mL). This indicates that peak exposure occurred shortly after dosing, and the nasal concentrations were reduced by 3 h post-dose. There was minimal intranasal accumulation upon repeated dosing, as the concentrations of IN-006 measured 22 h after a single dose were <2% of the concentrations immediately following dosing (Fig. 2). Though a very rough estimate, the difference in nasal concentrations between 30 min and 22 h post-dose suggest the interval represented ~4–9 half lives, which would be consistent with an intranasal half-life on the roughly estimated order of 2–6 h, markedly longer than the timescale of mucociliary clearance transit time estimates of ~5–15 min from saccharin transit time tests.[16,17]

Serum concentrations of IN-006 were detectable by 12 h following nebulization at the 90 mg dose and continued to rise through 120 h after a single dose (cohorts 1 and 2), or through 216 h following the first dose in those who received multiple doses (cohort 3) (Fig. 3). In the multiple dose cohort, Cmax in the serum occurred following the final dose, indicating accumulation. The elimination half-life of IN-006 in the serum was estimated to be ~253, 292, and 402 h in the 30 mg single dose, 90 mg single dose, and the 7 daily 90 mg dose cohorts respectively, comparable with regdanvimab following intravenous administration (288 h[18]) (Table 4). Although the serum concentrations of IN-006 were markedly lower than those in the nasal fluid (Serum geometric mean C_{max} of 0.51 μg/mL, compared to 740 μg/mL in nasal fluid), the serum concentration remained well above the IC_{50} range achievable by potent antiviral mAbs (e.g., IC_{50} of motavizumab and nirsevimab against RSV ranged between 0.5 and 60 ng/mL for most viral isolates.[19,20]

Discussion

Longstanding dogma has suggested that it is highly challenging to stably nebulize mAbs,[21–24] and that biologic drugs would be quickly eliminated from the respiratory tract either by systemic absorption, physical mucociliary clearance, or degradation by alveolar macrophages, making it difficult to sustain therapeutic concentrations.[25,26] However, in this Phase 1 study, we found that IN-006, a reformulation of regdanvimab for nebulized delivery at point of care, was safe and well tolerated in healthy adults. High concentrations of drug were recovered from nasal samples, and detectable levels were present in the serum. We also found that the treatment was easily self-administered by participants and was completed within minutes, with minimal side effects.

Subjects were asked to breathe in slowly from the nebulizer through their mouth and out quickly through their nose. For this reason, the observed nasal swab concentrations of IN-006 represent the fraction of drug that was not deposited in the lower respiratory tract upon inhalation, and instead was deposited in the nasal turbinates during nasal exhalation. Nasal delivery upon nasal exhalation was visually apparent in pilot experiments: whereas there was no visible aerosol plume upon nasal exhalation, oral exhalation led to a clearly visible plume of aerosol. Despite not directly inhaling into the nose, the amount of IN-006 deposited in the nasal passages during exhalation was well above typical antiviral mAb IC_{50s}, even 22–24 h after dosing. Indeed, in the multiple dose cohort, the mAb concentrations achieved ranged from 3–7 orders of magnitude higher than the IC_{50} against susceptible variants (~4–20 ng/mL,[13,27]). This almost certainly reflects the much smaller surface area of the nasal turbinates relative to the conducting airways and the deep lungs, which require far less total mAb dosed to achieve very high concentrations. The ability to achieve very high mAb concentrations in the nasal turbinates also strongly supports a once-daily dosing regimen, or twice-daily dosing regimens with a lower inhaled dose. Since SARS-CoV-2 infection and replication initiates in the upper respiratory tract, these data suggest that inhaled delivery of a neutralizing mAb

Cohort	Day	Median (range)	Intranasal swab geometric mean (% Geometric CV)											
		t_{max} (hr)	C_{max} (ng/mL)	C_{max}/D (ng/mL/mg)	$AUC_{0\text{-}t\text{last}}$ (hr*ng/mL)	$AUC_{0\text{-}t\text{last}}$/D (hr*ng/mL/mg)	$AUC_{0\text{-}24\ h}$ (hr*ng/mL)	$AUC_{0\text{-}24\ h}$/D (hr*ng/mL/mg)	$AUC_{0\text{-}inf}$ (hr*ng/mL)	$AUC_{0\text{-}inf}$/D (hr*ng/mL/mg)	$t_{1/2}$ (hr)	R_{AUC}[e]	R_{AUC}[f]	R_{Cmax}
SAD cohort 1 30 mg IN-006, n = 5	1	3.1 (3.1–3.2)	146.2 (167%)	4.9 (167%)	520 (254%)	17.4 (254%)	1770 (166%)	59.0 (166%)	1354[a] (105%)	45.1[a] (105%)	2.9[a] (14%)	NA	NA	NA
SAD cohort 2 90 mg IN-006, n = 6	1	3.1 (3.1–3.1)	458.7 (111%)	5.1 (111%)	4294 (201%)	47.7 (201%)	6041 (104%)	67.1 (104%)	7204[b] (108%)	80.1[b] (108%)	6.0[b] (77%)	NA	NA	NA
MD cohort 3 90 mg IN-006 once daily, n = 6	1	0.6 (0.6–0.6)	739.8 (83%)	8.2 (83%)	1333 (2007%)	14.8 (2007%)	8211 (84%)	91.2 (84%)	11,627[c] (76%)	129[c] (76%)	3.1[c] (2.4%)	NA	NA	NA
MD cohort 3 90 mg IN-006 once daily, n = 6	7	3.1 (3.1–3.1)	264.6 (141%)	2.9 (141%)	1523 (338%)	16.9 (338%)	2943 (141%)	32.7 (141%)	3334[d] (83%)	37.0[d] (83%)	2.5[d] (20%)	0.3[c] (144%)	2.2 (1978%)	0.4 (106%)

NA, Not applicable. [a]n = 2. [b]n = 5. [c]n = 3. [d]n = 4. [e]R_{AUC} calculated as AUC_{tau} Day 7/$AUC_{0\text{-}inf}$ Day 1. [f]R_{AUC} calculated as AUC_{tau} Day 7/$AUC_{0\text{-}t\text{last}}$ Day 1.

Table 3: **Summary of key intranasal PK parameters by cohort and dose day.**

could enable effective treatment for mild to moderate COVID-19 to reduce risk of progression to severe COVID-19.

Although mAbs were proven to be effective therapeutics against sensitive strains of SARS-CoV-2, the necessity for administration by IV, IM, or SC routes substantially restricted the scope of their use in clinical practice. Further, the requirement for infusion centers and post-dosing observation both severely limited the number of patients that could be treated and also greatly increased costs and overall burden on the healthcare system.[8,9] In contrast, nebulized delivery with a handheld nebulizer enables the convenience of at-home dosing and takes only minutes to complete. IM injections are limited by the volume that can be administered per injection (~1–3 mL),[28] which in turn limits the total dose of mAb that can be given at a time. Furthermore, IV, IM, and SC routes achieve Cmax pf mAb in the airway lining fluid only after a delay of one or more days, and, even then, only achieve airway concentrations that are a fraction of the concentrations in plasma.[10,11,29] The inability to achieve high concentrations of mAb in the respiratory tract after systemic dosing is likely partly responsible for the failure of many prior clinical studies of intravenously administered mAbs for ARIs. For instance, in a clinical trial of the anti-influenza mAb CR6261, the peak nasal concentration was not achieved until 2 days after IV infusion,[12] and the peak nasal concentration of ~0.597 μg/mL was still ~10-fold lower than the concentrations we observed in this study for IN-006 at the trough after inhaled dosing (~5.8 μg/mL), despite the much lower dose administered (90 mg IN-006 vs. ~3000+ mg CR6261[12]). Unsurprisingly, patients were not protected against influenza in that study of CR6261 and development was halted.[30] Notably, these data suggest that nebulized dosing of mAb provides not only higher concentrations in the respiratory fluids, at a faster speed compared to systemic dosing, but can do so with far greater delivery efficiency. That greater delivery efficiency enables a lower total dose of mAb per patient, reducing costs of treatment and increasing the number of patients that could be treated from a fixed supply of mAb. Combining these factors, we believe that inhalation will become the preferred route of mAb delivery for treating ARIs.

Serious disease due to SARS-CoV-2 is accompanied by the spread of the virus from the site of the initial upper respiratory tract infection to the deep lungs.[7] Unfortunately, the exact timing of such spread varies across individuals, and some data suggest viruses can even reach the LRT during the early stages of disease when symptoms first emerge.[31,32] Thus, we believe dosing to both the URT and LRT, rather than focusing exclusively on the URT (e.g., via nasal sprays), will be important to reduce risk of COVID-induced pneumonia and hospitalization. While IN-006 levels in the LRT were

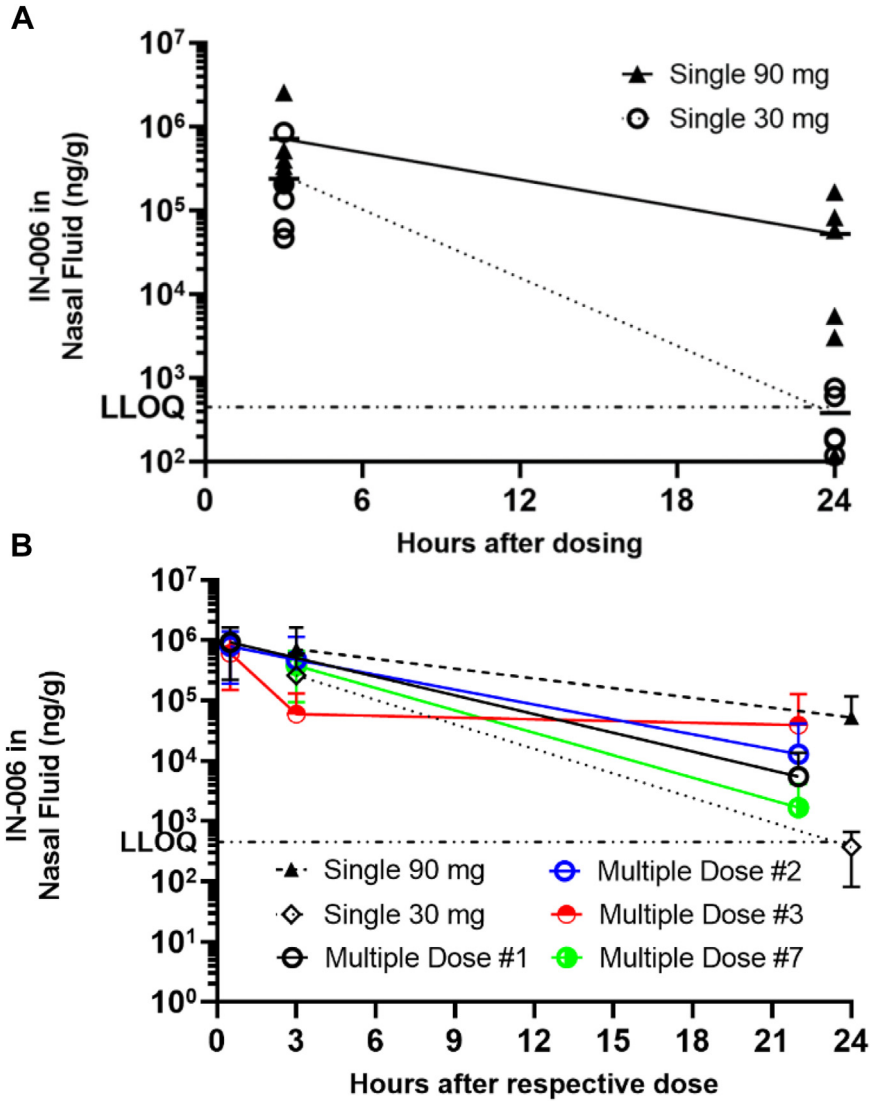

Fig. 2: **(A)** Nasal fluid concentrations in single dose cohorts. **(B)** Comparison of nasal concentrations between single dose and multiple dose cohorts at varying times after each dose. Lower limit of quantitation (LLOQ) varied by sample, depending on the mass of nasal fluid collected on swab. For this figure, for samples that were measured as below the limit of quantitation (BLQ), the concentration was reported as the midway point between 0 and the LLOQ for the purpose of calculating group average concentrations at each timepoint. For example, a BLQ sample with an LLOQ of 1000 ng/mL was reported at 500 ng/mL. Error bars represent standard deviation. For panel B, please refer to online Supplemental Figure S1 for further details and for an updated version of this figure in which two datapoints suspected to be swapped at the time of data collection (one individual in Multiple Dose 3, at 3 h and 22 h) were corrected. Data here in Panel B is are not corrected for this suspected error.

not directly measured in this study, the appreciable serum concentrations and the delayed serum Tmax both strongly suggest we were efficiently delivering IN-006 into the LRT and the deep lungs. Indeed, in a toxicokinetic multiple dose nebulization study of IN-006 in rats, airway fluid concentrations exceeded the serum concentrations by ~100-fold. Efficient delivery into the LRT is a direct consequence of our design requirement for the vibrating mesh nebulizer. The droplet size distribution generated by the nebulizer (the fine particle fraction, i.e., droplets <5 μm, and particularly those <2.5 μm[14]) was intentionally selected to deliver a portion of the mAbs throughout the LRT and deep lung. Furthermore, the fact that we observed a slow, steady rise of serum concentrations in single dose cohorts over ~4 days, and peak serum concentrations in the multiple dose cohort 2 days after last dose, suggests that we may be sustaining high levels of IN-006 in the deep lungs for

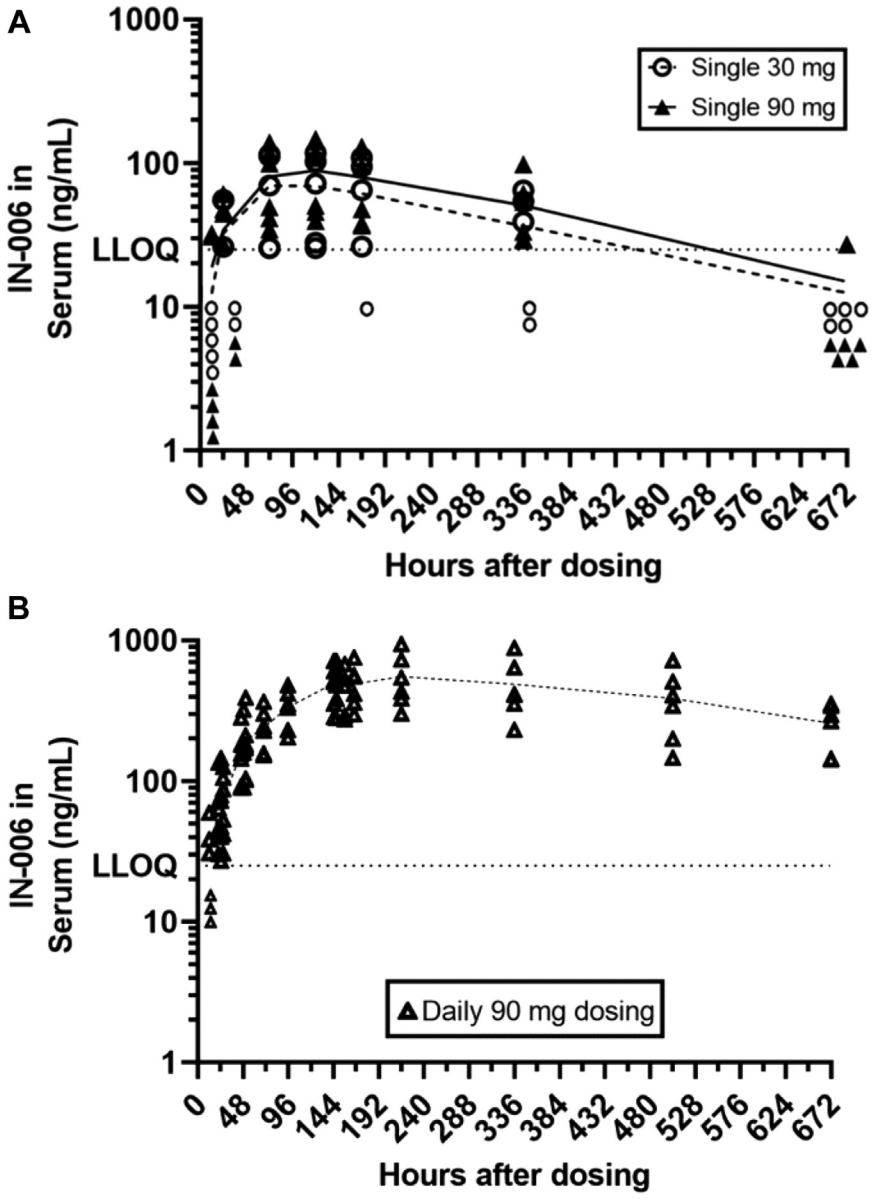

Fig. 3: Serum IN-006 concentrations in **(A)** single dose cohorts, and **(B)** multiple dose cohort (last dose administered at 144 h). Symbols plotted below the dashed LLOQ line at 25 ng/mL represent the number of samples in each group that were BLQ at each timepoint.

at least 2–4 days after each dose. Assuming a ratio of roughly 100:1 of antibody concentrations in the airway fluid:serum, the mean serum concentration of 0.51 μg/mL at Day 9 should translate to pulmonary fluid concentrations on the order of 51 μg/mL. These very high mAb levels may reduce the risk of viral escape and suggest that shorter durations of therapy may afford appreciable protection against hospitalization.

The single- and multi-dose groups yielded somewhat different estimates for the serum terminal half-life, with the single-dose estimates of ~253 and 292 being more similar to the 288 h expected from prior studies of IV-administered regdanvimab.[18] The estimate of 402 h for the multi-dose group may be confounded by continued distribution of IN-006 from the deep lung into the systemic circulation (i.e., an extended alpha phase of distribution), potentially contributing toward a slower estimated elimination. For the single-dose cohorts, the estimation of serum half-life was somewhat complicated by a number of samples that were BLQ at the last timepoint collected. Nonetheless, we expect the serum half-life of IN-006 that distributes into the serum to be

Cohort	Day	Median (range)	Serum geometric mean (% Geometric CV)											
		t_{max} (hr)	C_{max} (ng/mL)	C_{max}/D (ng/mL/mg)	$AUC_{0\text{-tlast}}$ (hr*ng/mL)	$AUC_{0\text{-tlast}}$/D (hr*ng/mL/mg)	$AUC_{0\text{-}24\ h}$ (hr*ng/mL)	$AUC_{0\text{-}24\ h}$/D (hr*ng/mL/mg)	$AUC_{0\text{-inf}}$ (hr*ng/mL)	$AUC_{0\text{-inf}}$/D (hr*ng/mL/mg)	$t_{1/2}$ (hr)	R_{AUC}[c]	R_{AUC}[d]	R_{Cmax}
SAD cohort 1 30 mg IN-006, n = 5	1	121.5 (72–122)	58.6 (86%)	2.0 (86%)	9714 (298%)	324 (298%)	8.4[b] (NR)	0.3[b] (NR)	50,837[a] (19%)	1695[a] (19%)	252[a] (15%)	NA	NA	NA
SAD cohort 2 90 mg IN-006, n = 6	1	118.9 (116–121)	77.0 (66%)	0.9 (66%)	20,590 (102%)	229 (102%)	368[b] (79%)	4.1[b] (79%)	70,041[a] (31%)	778[a] (31%)	287[a] (28%)	NA	NA	NA
MD cohort 3 90 mg IN-006 once daily, n = 6	1	22 (22–22)	59.0 (58%)	0.7 (58%)	408 (105%)	4.5 (105%)	NC	NC	NC	NC	NC	NA	NA	NA
MD cohort 3 90 mg IN-006 once daily, n = 6	7	44.4 (0.6–96.5)	539.9 (44%)	6.0 (44%)	206,699 (44%)	2297 (44%)	10,878 (35%)	121 (35%)	297,136[a] (62%)	3302[a] (62%)	234[a] (15%)	NC	26.7 (89%)	9.1 (51%)

NA, Not applicable; NC, Not calculated; NR, Not reported. [a]n = 2. [b]n = 4. [c]R_{AUC} calculated as AUC_{tau} Day 7/$AUC_{0\text{-inf}}$ Day 1. [d]R_{AUC} calculated as AUC_{tau} Day 7/$AUC_{0\text{-tlast}}$ Day 1.

Table 4: **Summary of key serum PK parameters by cohort and dose day.**

no different than if the mAb was administered systemically, and to provide effective protection against systemic viremia, in the unlikely situation that systemic viremia occurs.

Regdanvimab (administered IV) was shown to be highly efficacious for preventing severe COVID-19 in a global Phase 3 study, leading to its approval in Republic of Korea and European Union (EMEA/H/C/005854) for preventing severe disease in patients presenting with mild to moderate COVID-19, and emergency use authorization (EUA) or conditional marketing authorization in several additional countries worldwide. Nevertheless, as SARS-CoV-2 continues to evolve, we are pursuing development of a separate inhaled biologic that possesses potent binding activity against every variant tested to date. In light of the surprisingly long airway retention of IN-006 observed here, we may reduce the duration of dosing from 7 days to 5 days in future clinical studies.

Compared to placebo, there appeared to be more IN-006 treated subjects who experienced a cough, oropharyngeal discomfort, or throat irritation. This could be due to the very small number of participants, particularly in the placebo group. Importantly, each of these AEs were mild, resolved spontaneously, and there was no evidence of a dose-dependent effect. These findings underscore the likely safety of inhaling antiviral mAbs, particularly on balance against the risks associated with untreated ARIs, a balance that we will verify in future clinical studies. The transaminase elevation observed in one participant in the 30 mg single dose cohort was judged to not be treatment-related by the Study PI, primarily due to the long interval (28 days) between dosing and the observed elevation, as well as the intervening normal transaminases observed at 7 days after dosing. Further, this was not observed in any subjects in the multiple-dose, high-dose groups. Aside from those TEAEs described in Table 2 and in the Discussion, other values for patients were within normal ranges. Notably, these safety assessments were conducted in generally healthy individuals. Although it remains to be seen whether patients infected with SARS-CoV-2 would see similarly good safety and tolerability as healthy subjects, our intended time for treatment is in the outpatient setting, as soon as possible after COVID-19 diagnosis, and prior to the need for supplemental oxygen or hospitalization. As such, we expect these patients would be relatively similar to healthy subjects enrolled in this Phase 1 study. We also wish to point out that our method of inhaled delivery relies on oral inhalation rather than nasal inhalation. Thus, delivery to the lower respiratory tract is not affected by potential infection-induced variations in the upper respiratory tract.

Our study had several limitations. The population we studied was small, as is typical of first-in-human studies. Moreover, only healthy adults were enrolled in our study, limiting its generalizability. Determining the full

safety profile of nebulized IN-006 will require additional studies in larger numbers of more diverse individuals. The low recovered sample mass from nasal swabs required substantial dilution for sample recovery, limiting the sensitivity of the assay. As a result, some 24-h post-dose samples and all later nasal samples were below the limit of quantification. Some eligibility criteria were modified during the study. Originally, we required that subjects have FEV_1 of ≥90% predicted. In practice, we found that many would-be subjects fell just short of the 90% criterion despite being otherwise healthy. Since relaxing this enrollment criterion did not impact our ability to assess for safety, we relaxed the threshold to ≥80%, which we believe may provide a better representation of the range of FEV_1s that might be present in the treatable patient population. Another limitation of our study is the lack of direct measurement of drug levels in the LRT, due to COVID pandemic protocols that prohibited collection of BALF at the time of this study. The gradual increase in serum drug levels over many days led us to speculate that a single inhaled dose may sustain therapeutic levels of mAbs in the LRT for many days to weeks. We are now conducting a Phase 1 b study in which we will be able to collect BALF samples to provide direct measurement of the levels of IN-006 in the LRT. A better understanding of the efficiency of LRT delivery as well as LRT PK will better help guide future dosing regimens of other inhaled mAb therapies, including potential use and limits for prophylaxis for immunocompromised individuals or those with high-risk exposure (e.g., a transcontinental plane ride, infectious disease ward in hospital during the winter season).

In conclusion, IN-006, a reformulation of regdanvimab for inhaled delivery, was found to be safe and well-tolerated in healthy participants at single doses of 30 mg and 90 mg, as well as seven consecutive daily doses of 90 mg. Nebulization resulted in IN-006 levels in nasal fluids, and likely the lungs, that were orders of magnitude above the inhibitory concentrations of sensitive SARS-CoV-2 variants within 30 min, and the continued rise of serum concentration for days after dosing implied substantial lasting IN-006 levels in the lungs. These data support the ongoing development of inhaled antiviral mAbs for ARIs.

Contributors

TRM, MH, MB, JH and SKL conceptualized and designed the study.

LB, BF, MDM, HK, YP, and ZR performed experiments. JMC, SYL, BF, and JH supervised experiments.

LB, BF, MDM, MH, JH, and SKL contributed to data analysis.

BF, LB, TRM, and MM accessed and verified the underlying data.

JDL, FF, TRM, and JH managed & coordinated the clinical trial. JDL was Principal Investigator.

JH, SKL, and JBW acquired the financial support.

All authors critically revised the manuscript and approved the submitted version.

The academic authors and the authors who are employees of the sponsor vouch for the accuracy and completeness of the data, the statistical analysis, and the fidelity of the study to the protocol.

Data sharing statement

Deidentified data from this study will be made available from the corresponding author upon reasonable request. Reagents related to this study may be available upon reasonable request submitted to the corresponding author, pending availability. Some reagents associated with this study, including IN-006, are no longer clinically available and therefore cannot be shared.

Declaration of interests

TM, LB, BF, MH, MB, MM, ZR, JW, FF, and JH are employees of Inhalon Biopharma/Mucommune and may hold shares in Inhalon Biopharma, Inc. HK, YP, CK, JMC, and SYL are employees of Celltrion, Inc, which received funding from the government of Korea to advance Regdanvimab, which was used to cover salary for their work on this project. JL received partial salary support for his work on this project. SKL is founder of Mucommune, LLC and currently serves as its interim CEO. SKL is also founder of Inhalon Biopharma, Inc, and currently serves as its CSO as well as on its Board of Director and Scientific Advisory Board. S.K.L has equity interests in both Mucommune and Inhalon Biopharma; S.K.L's relationships with Mucommune and Inhalon are subject to certain restrictions under University policy. The terms of these arrangements are managed by UNC-CH in accordance with its conflict of interest policies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105582.

References

1 Pinto AL, Rai RK, Brown JC, et al. Ultrastructural insight into SARS-CoV-2 entry and budding in human airway epithelium. *Nat Commun*. 2022;13(1):1609.
2 Lee IT, Nakayama T, Wu C-T, et al. ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs. *Nat Commun*. 2020;11(1):5453.
3 Jia HP, Look DC, Shi L, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *J Virol*. 2005;79(23):14614–14621.
4 Zhang L, Peeples ME, Boucher RC, Collins PL, Pickles RJ. Respiratory syncytial virus infection of human airway epithelial cells is polarized, specific to ciliated cells, and without obvious cytopathology. *J Virol*. 2002;76(11):5654–5666.
5 Sims AC, Burkett SE, Yount B, Pickles RJ. SARS-CoV replication and pathogenesis in an in vitro model of the human conducting airway epithelium. *Virus Res*. 2008;133(1):33–44.
6 Zhang L, Bukreyev A, Thompson CI, et al. Infection of ciliated cells by human parainfluenza virus type 3 in an in vitro model of human airway epithelium. *J Virol*. 2005;79(2):1113–1124.
7 Hou YJ, Okuda K, Edwards CE, et al. SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. *Cell*. 2020;182(2):429–446.e14.
8 Lai SK, McSweeney MD, Pickles RJ. Learning from past failures: challenges with monoclonal antibody therapies for COVID-19. *J Control Release*. 2021;329:87–95.

9 Cruz-Teran C, Tiruthani K, McSweeney M, Ma A, Pickles R, Lai SK. Challenges and opportunities for antiviral monoclonal antibodies as COVID-19 therapy. *Adv Drug Deliv Rev*. 2021;169:100–117.
10 Hart TK, Cook RM, Zia-Amirhosseini P, et al. Preclinical efficacy and safety of mepolizumab (SB-240563), a humanized monoclonal antibody to IL-5, in cynomolgus monkeys. *J Allergy Clin Immunol*. 2001;108(2):250–257.
11 Dall'Acqua WF, Kiener PA, Wu H. Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). *J Biol Chem*. 2006;281(33):23514–23524.
12 Han A, Czajkowski L, Rosas LA, et al. Safety and efficacy of CR6261 in an influenza A H1N1 healthy human challenge model. *Clin Infect Dis*. 2020;73(11):e4260–e4268.
13 van der Straten K, van Gils MJ, de Taeye SW, de Bree GJ. Optimization of anti-SARS-CoV-2 neutralizing antibody therapies: roadmap to improve clinical effectiveness and implementation. *Front Med Technol*. 2022;4:867982.
14 Darquenne C. Aerosol deposition in health and disease. *J Aerosol Med Pulm Drug Deliv*. 2012;25(3):140–147.
15 McSweeney MD, Stewart I, Richardson Z, et al. Stable nebulization and muco-trapping properties of regdanvimab/IN-006 support its development as a potent, dose-saving inhaled therapy for COVID-19. *Bioeng Transl Med*. 2022;8:e10391.
16 Emma R, Caponnetto P, Cibella F, et al. Short and long term repeatability of saccharin transit time in current, former, and never smokers. *Front Physiol*. 2020;11:1109.
17 Rodrigues F, Freire AP, Uzeloto J, et al. Particularities and clinical applicability of saccharin transit time test. *Int Arch Otorhinolaryngol*. 2019;23(2):229–240.
18 Syed YY. Regdanvimab: first approval. *Drugs*. 2021;81(18):2133–2137.
19 Wu H, Pfarr DS, Johnson S, et al. Development of motavizumab, an ultra-potent antibody for the prevention of respiratory syncytial virus infection in the upper and lower respiratory tract. *J Mol Biol*. 2007;368(3):652–665.
20 Zhu Q, McLellan JS, Kallewaard NL, et al. A highly potent extended half-life antibody as a potential RSV vaccine surrogate for all infants. *Sci Transl Med*. 2017;9(388):eaaj1928.
21 Respaud R, Vecellio L, Diot P, Heuze-Vourc'h N. Nebulization as a delivery method for mAbs in respiratory diseases. *Expet Opin Drug Deliv*. 2015;12(6):1027–1039.
22 Mayor A, Thibert B, Huille S, Respaud R, Audat H, Heuzé-Vourc'h N. Inhaled antibodies: formulations require specific development to overcome instability due to nebulization. *Drug Deliv Transl Res*. 2021;11(4):1625–1633.
23 Bodier-Montagutelli E, Respaud R, Perret G, et al. Protein stability during nebulization: mind the collection step. *Eur J Pharm Biopharm*. 2020;152:23–34.
24 Bodier-Montagutelli E, Mayor A, Vecellio L, Respaud R, Heuzé-Vourc h N. Designing inhaled protein therapeutics for topical lung delivery: what are the next steps? *Expet Opin Drug Deliv*. 2018;15(8):729–736.
25 Loira-Pastoriza C, Todoroff J, Vanbever R. Delivery strategies for sustained drug release in the lungs. *Adv Drug Deliv Rev*. 2014;75:81–91.
26 Suri R. The use of human deoxyribonuclease (rhDNase) in the management of cystic fibrosis. *BioDrugs*. 2005;19(3):135–144.
27 Liu L, Iketani S, Guo Y, et al. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature*. 2022;602(7898):676–681.
28 Nagy CF, Leach TS, King A, Guttendorf R. Safety, pharmacokinetics, and immunogenicity of obiltoxaximab after intramuscular administration to healthy humans. *Clin Pharmacol Drug Dev*. 2018;7(6):652–660.
29 McSweeney M, Stewart I, Richardson Z, et al. Stable nebulization and muco-trapping properties of Regdanvimab/IN-006 supports its development as a potent, dose-saving inhaled therapy for COVID-19. *bioRxiv*. 2022. https://doi.org/10.1101/2022.02.27.482162.
30 Throsby M, van den Brink E, Jongeneelen M, et al. Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells. *PLoS One*. 2008;3(12):e3942.
31 Chen PZ, Bobrovitz N, Premji ZA, Koopmans M, Fisman DN, Gu FX. SARS-CoV-2 shedding dynamics across the respiratory tract, sex, and disease severity for adult and pediatric COVID-19. *Elife*. 2021;10:e70458.
32 Lim A-Y, Cheong H-K, Oh YJ, et al. Modeling the early temporal dynamics of viral load in respiratory tract specimens of COVID-19 patients in Incheon, the Republic of Korea. *Int J Infect Dis*. 2021;108:428–434.