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Pharmacokinetics and pharmacodynamics of inhaled molgramostim in healthy people

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ABSTRACT

Background Inhaled molgramostim, a form of recombinant human granulocyte–macrophage colony stimulating factor (GM-CSF), is a promising investigational pharmacotherapy for autoimmune pulmonary alveolar proteinosis (aPAP); however, its pharmacology in healthy subjects has not been reported.

Methods This randomised, double-blind, placebo-controlled, single-centre, phase 1 clinical trial assessed the safety, tolerability, pharmacokinetics and pharmacodynamics of inhaled molgramostim in healthy adults in single ascending dose (SAD) and multiple ascending dose (MAD) studies: one 150, 300 or 600 µg administration or six consecutive daily 300 or 600 µg administrations with evaluations over 28 or 34 days, respectively. The primary endpoint was safety, which was evaluated based on the number and severity of treatment-emergent AEs following single and multiple inhaled doses of molgramostim.

Results 42 subjects were enrolled including 18 in the SAD study and 24 in the MAD study; all completed the study. Inhaled molgramostim in healthy people was well tolerated and no dose-limiting safety concerns or anti-drug antibody formation were observed in either study. GM-CSF was measurable in serum 30 min after administration of inhaled molgramostim, peaked at 2 hours for all three doses and had an elimination half-life of 1.7±0.0 to 5.9±0.9 hours in the SAD and MAD studies. Systemic GM-CSF exposure was non-linear in both the SAD and MAD studies. Inhaled molgramostim caused a rapid increase in white blood cells (WBC) counts and leucocyte subsets that normalised by 8 hours (SAD) or 15–21 days (MAD). Fractional exhaled nitric oxide remained within the normal range at all doses but was numerically, but not significantly, increased at the 600 µg dose.

Conclusions In healthy people, inhaled molgramostim was well-tolerated and resulted in systemic exposure at picogram levels, which had the expected PD effects on blood leucocyte levels that mostly remained within normal ranges.

Trial registration number NCT02468908; EudraCT No. 2013-001687-32

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Inhaled granulocyte–macrophage colony stimulating factor (GM-CSF) has been shown to be effective and well tolerated for the treatment of autoimmune pulmonary alveolar proteinosis (aPAP).

WHAT THIS STUDY ADDS

⇒ This is the first pharmacokinetic (PK) and pharmacodynamic (PD) study of the inhaled form of recombinant GM-CSF; previous studies have only evaluated the PK and PD of intravenous and subcutaneous recombinant GM-CSF.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The PK/PD results from this study will help inform users of inhaled recombinant GM-CSF for patients with aPAP of its low systemic exposure and resulting low PD effects.

INTRODUCTION

Endogenous granulocyte-macrophage colony stimulating factor (GM-CSF) is a polypeptide hormone normally produced by multiple cell types that participates in haematopoiesis,[1] innate and adaptive immunity,[2] and is required by alveolar macrophages to effectively clear surfactant from the lung surface.[3–5] Recombinant GM-CSF (rGM-CSF) has been produced pharmacologically[6] and administered systemically to treat haematologic diseases[7 8], and it transiently increases the numbers of white blood cells (WBCs) and leucocyte subsets after systemic administration in healthy individuals.[9 10]

Autoimmune pulmonary alveolar proteinosis (aPAP) is a rare lung disease caused by disruption of GM-CSF signalling by GM-CSF autoantibodies and is characterised by progressive dyspnoea and hypoxaemia from excessive accumulation of surfactant within pulmonary alveoli.[11–16] Without GM-CSF, alveolar macrophages cannot effectively clear surfactant, which accumulates in the endoalveolar space and impairs oxygen delivery to the blood.[17] Currently, aPAP is treated by whole lung lavage,[18 19] a procedure requiring hospitalisation, general anaesthesia, simultaneous mechanical ventilation of the untreated lung and repeated cycles of saline infusion and drainage accompanied

by chest percussion to physically 'wash' excess surfactant out of the lung. Inhaled rGM-CSF is a promising investigational pharmacologic therapy for aPAP.[20–26] Studies have documented the efficacy and safety of inhaled rGM-CSF as therapy for aPAP,[27–29] and a recent meta-analysis suggests that nebulisation is a superior route of administration at inducing remission and improving gas transfer in patients with aPAP compared with the subcutaneous route.[26 30]

While the pharmacology of rGM-CSF after systemic administration has been studied,[31] data on the pharmacokinetics (PK) and pharmacodynamics (PD) of inhaled rGM-CSF are lacking in published literature. Therefore, we conducted a randomised, placebo-controlled, blinded, phase 1 clinical trial in healthy people to determine the safety, PK and PD following single or repeated administration(s) of ascending doses of molgramostim inhalation solution. An abstract of this study was previously presented at the Annual Meeting of the American Thoracic Society.[32]

METHODS AND MATERIALS

Participants

Eligible participants were women or men, 18 to 55 years of age, life-long non-smokers with no history of nicotine-containing product use, a body mass index of ≥18.5 and ≤32.0 kg/m^2 and healthy without clinically significant historical, physical, laboratory, spirometric or electrocardiographic abnormalities at enrolment. Women were required to be of non-childbearing potential.

Trial Oversight

The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines with periodic review by an independent data and safety monitoring committee. The protocol was designed by the sponsor (Savara ApS, a subsidiary of Savara Inc.) and was approved by the Medicines and Healthcare Products Regulatory Agency, United Kingdom (UK) and Office for Research Ethics Committees, Northern Ireland, UK. The sponsor collected and analysed the data. The authors had access to the data, vouched for the accuracy and completeness of the data and the fidelity of trial conduct to the protocol, and wrote the manuscript.

Patient and Public Involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Trial Design and Treatments

This was a randomised, double-blind, placebo-controlled, single-centre, phase 1 clinical trial conducted in healthy people. The trial was conducted at Celerion, Inc., Belfast, Northern Ireland, UK and consisted of single ascending dose (SAD) and multiple ascending dose (MAD) studies (online supplemental figure S1). The primary endpoint of the trial was to assess the safety of inhaled molgramostim in healthy adults following single and multiple ascending doses. The secondary endpoints were to assess the PK of inhaled molgramostim in healthy adults following single and multiple ascending doses. Exploratory endpoints were to assess the effects of molgramostim on the development of antibodies to GM-CSF, WBC counts, fractional exhaled nitric oxide (FeNO) and the time from the start of the Q wave to the end of the T wave (QT)/corrected QT (QTc) interval on electrocardiogram.

In the SAD study, subjects were randomly assigned in a 1:1:1:1.5 ratio to receive one administration of inhaled molgramostim (150, 300 or 600 µg) or matching placebo. In the MAD study, subjects were randomly assigned in a 1.5:1.5:1 ratio to receive six once-daily administrations of inhaled molgramostim (300 or 600 µg) or matching placebo.

Molgramostim (Savara ApS, a subsidiary of Savara Inc.) is a non-glycosylated rGM-CSF produced using recombinant DNA technology via a bacterial (*Escherichia coli*) expression system. The molgramostim inhalation solution drug product contained 250 µg/mL rGM-CSF. The placebo product was molgramostim inhalation solution but without the 250 µg/mL rGM-CSF. Excipients in the drug product and in the placebo product were mannitol, polyethylene glycol 4000 (PEG 4000), recombinant human albumin, disodium phosphate (anhydrous), citric acid (monohydrate) and water for injection. Interventions were administered with the use of an ultrasonic, vibrating mesh nebuliser (eFlow, PARI Pharma). Details regarding the trial site, design, and management are provided in the supplemental file.

Safety Assessments

Safety was evaluated based on the number, severity and relatedness of adverse events (AEs) arising from medical history, physical examinations, routine laboratory tests, pulmonary function tests, electrocardiography and assessment of serum anti-GM-CSF antibodies.

PK Assessments

All PK parameters were derived using non-compartmental analysis conducted in Phoenix WinNonlin (Version 6.3), in accordance with standardised approaches outlined in United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) regulatory guidance, including the FDA's *Guidance for Industry: Bioanalytical Method Validation* and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) M3(R2). While these methods are standard in early-phase clinical trials, a brief description of key parameters and their clinical relevance has been included to aid interpretation by a broader clinical audience.

Outcome measures included pre-administration and post-administration serum GM-CSF levels and

non-compartmental analysis to determine PK parameters (online supplemental table S1) including area under the curve (AUC) of serum GM-CSF concentration versus time from administration to the last measured concentration (AUC_{last}), AUC from time zero to infinity ($AUC_{0\text{-}inf}$), AUC extrapolated as a percentage of the total ($AUC_{\%extrap}$), highest serum concentration (C_{max}), time to highest serum concentration (t_{max}), fraction of GM-CSF eliminated (k_{el}), and serum half-life ($t_{1/2}$), and AUC from time 0 to the dosing interval time for the final 24-hour period (AUC_{tau}), maximal observed serum GM-CSF concentration following multiple doses at steady state ($C_{max,ss}$), minimum observed/measured non-zero serum concentration at steady state ($C_{min,ss}$), average multiple-dose serum GM-CSF concentration calculated as AUC_{tau}/τ at steady state, where τ=24 hours ($C_{avg,ss}$), trough serum drug concentration (observed at the end of a dosing interval) measured on days 4, 5 and 6 (C_{trough}), time of maximum measured serum GM-CSF concentration following multiple doses at steady state ($t_{max,ss}$), time of minimum measured serum GM-CSF concentration sampled during a dosing interval ($t_{min,ss}$), ratio of the magnitude of change between the maximum observed/measured concentrations relative to the minimum observed concentration at steady state (Swing) and total body clearance at steady state (CL_{ss}/F). GM-CSF was measured by a validated ELISA at IPM Biotech (Hamburg, Germany), which detects both endogenous glycosylated GM-CSF and pharmacologic, non-glycosylated rGM-CSF,[33] and had a lower limit of quantification (LLoQ) of 2.0 pg/mL.

PD Assessments

Outcome measures informing PD included pre-administration and post-administration enumeration of total WBC and subset counts and FeNO concentration.

Statistical Analysis

Numeric data were summarised using descriptive statistics. Comparisons of group means (parametric data), medians (non-parametric data), numbers and percentages (categorical data) were made using t-test, Mann-Whitney test or Fisher's exact test, as appropriate. All reported p-values were two-sided without adjustment for multiple testing. P-values <0.05 were considered to indicate statistical significance. Non-compartmental PK parameters were calculated using Phoenix WinNonlin (V. 6.3). Statistical comparisons were made using Prism (V. 10.1) (section 2, online supplemental file 1).

RESULTS

Participants

From May through September 2015, 42 healthy adults were enrolled and randomised to receive a single administration of inhaled molgramostim (150, 300 or 600 µg; n=4 each) or placebo (n=6) in the SAD study or inhaled molgramostim (300 or 600 µg; n=9 each) or placebo (n=6) daily for six consecutive days in the MAD study (online supplemental figure S2). Participants were blinded to the intervention. All subjects completed the assigned interventions and evaluations as planned (online supplemental tables S2 and S3). Baseline demographics and clinical characteristics of subjects are shown in table 1.

Safety of Inhaled Molgramostim

No serious AEs occurred during either SAD or MAD studies. In the SAD study, AEs occurred in 7 (58%) of 12 molgramostim-treated subjects, in a dose-independent fashion, and in 4 (67%) of 6 placebo-treated subjects (online supplemental table S4). Cough was the most common AE and occurred in 6 (50%) of 12 molgramostim-treated and 2 (33%) of 6 placebo-treated subjects (online supplemental table S4). Diarrhoea occurred in one molgramostim-treated and one placebo-treated subject (online supplemental table S4). Other AEs occurred in less than 10% of subjects. Of the 27 AE episodes that occurred during the SAD study, 4 (15%), 4 (15%) and 19 (70%) were judged, respectively, to be likely-related, possibly-related or unlikely-related to molgramostim administration (online supplemental table S4). In the MAD study, AEs occurred in 15 (83%) of 18 molgramostim-treated and 6 (100%) of 6 placebo-treated subjects (online supplemental table S5). Cough was most common and occurred in 15 (83%) of 18 molgramostim-treated and 6 (100%) of 6 placebo-treated subjects and was more common at the 600 µg than the 300 µg dose (online supplemental table S5). Headache occurred in 7 (39%) of 18 molgramostim- and 1 (17%) of 6 placebo-treated subjects (online supplemental table S5). Other AEs were less frequent and balanced among groups or occurred in fewer than 5% of subjects. Of the 226 AE episodes that occurred during the MAD study, 27 (12%), 11 (5%), 32 (14%), 145 (64%), and 11 (5%) were judged to be related, probably-related, possibly-related, unlikely-relatedss or unrelated, respectively, to molgramostim administration. Inhaled molgramostim had no effect on the electrocardiogram (online supplemental tables S6 and S7A-E). Changes in WBCs and leucocyte subsets remained mostly in the normal range. Anti-GM-CSF antibodies were not detected in any subject during the study. No other safety findings were noted for physical examination, vital signs, electrocardiograms, spirometry or laboratory findings. In both SAD and MAD studies, all AEs were considered mild and resolved without sequelae.

PK of Inhaled Molgramostim

SAD Study

Endogenous GM-CSF was not detected in the serum of any subjects before administration of any intervention or at any time in subjects receiving placebo. Following a single administration of inhaled molgramostim, rGM-CSF was rapidly absorbed and resulted in measurable rGM-CSF in serum by 0.5 hours with a T_{max} at 2 hours at all doses (figure 1, online supplemental tables S8 and

Table 1 Baseline Demographics and Clinical Characteristics of the Study Subjects

	Single ascending dose				Multiple ascending dose			
	Molgramostim Dose				Molgramostim Dose			**Overall N=42 (%)**
Characteristic	150 µg	300 µg	600 µg	Placebo	300 µg	600 µg	Placebo	
Gender, n (%) Female Male	 0 04 (100)	 0 04 (100)	 0 04 (100)	 0 06 (100)	 0 09 (100)	 1 (11) 8 (89)	 1 (17) 5 (83)	 2 (5) 40 (95)
Ethnicity, n (%) Hispanic or Latino	0	0	0	0	0	0	0	0
Race, n (%) Black White	0 04 (100)	1 (25) 3 (75)	0 04 (100)	0 06 (100)	1 (11) 8 (89)	0 09 (100)	0 06 (100)	2 (5) 40 (95)
Age* (years) Mean±SD Median (Min, Max)	n=4 43.8±10.1 44.0 (34, 53)	n=4 38.0±12.8 39.5 (21, 52)	n=4 38.3±12.8 39.0 (23, 52)	n=6 35.0±11.1 38.0 (18, 47)	n=9 32.0±11.5 30.0 (20, 51)	n=9 26.8±9.6 23.0 (20, 51)	n=6 32.8±13.4 29.5 (19, 53)	n=42 33.7±11.7 32.5 (18, 53)
Weight (kg) Mean±SD Median (Min, Max)	n=4 82.6±9.4 84.6 (69.8, 91.4)	n=4 78.0±12.2 74.4 (67.8, 95.2)	n=4 83.8±7.0 82.3 (77.0, 93.4)	n=6 82.6±11.8 80.8 (69.2, 103.6)	n=9 82.2±13.4 80.6 (70.6, 116.0)	n=9 79.1±9.3 76.8 (65.8, 92.6)	n=6 72.8±10.4 72.7 (58.2, 84.4)	n=42 80.0±10.8 79.0 (58.2, 116.0)
Height (cm) mean±SD Median (Min, Max)	n=4 172.5±5.8 172.0 (166, 180)	n=4 175.3±5.7 175.5 (168, 182)	n=4 174.5±6.4 172.0 (170, 184)	n=6 181.3±5.6 181.0 (175, 191)	n=9 177.3±7.1 176.0 (167, 193)	n=9 173.8±9.7 175.0 (152, 188)	n=6 174.0±7.6 175.5 (162, 182)	n=42 175.7±7.4 175.5 (152, 193)
BMI (kg/m^2) Mean±SD Median (Min, Max)	n=4 27.7±1.6 28.1 (25.3, 29.2)	n=4 25.3±2.8 25.3 (21.9, 28.7)	n=4 27.5±0.6 27.6 (26.6, 28.0)	n=6 25.0±2.2 25.2 (22.1, 28.4)	n=9 26.0±2.9 26.2 (21.8, 31.1)	n=9 26.4±4.0 26.2 (21.8, 31.7)	n=6 24.0±2.2 24.7 (20.4, 26.0)	n=42 25.9±2.8 25.6 (20.4, 31.7)

*Age at first dosing.
BMI, body mass index.

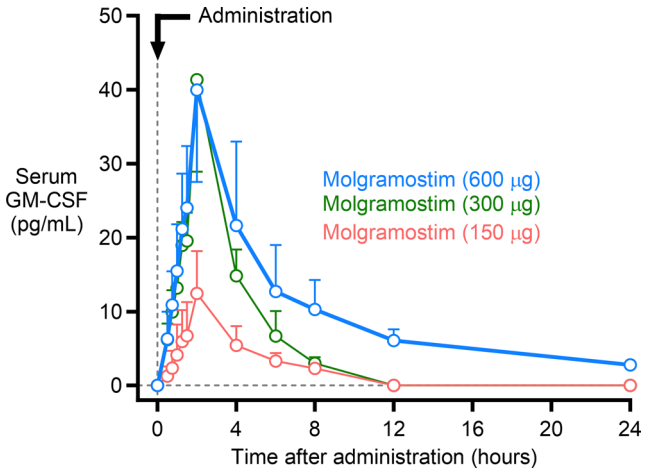

Figure 1 Serum GM-CSF concentration in the single ascending dose study. Twelve (n=4 per dose) healthy individuals received a single administration of inhaled molgramostim, and serum GM-CSF was measured at the indicated times (–0.25, 0.5, 0.75, 1, 1.25, 1.50, 2, 4, 6, 8, 12 and 24 hours). Symbols represent the mean value among four healthy subjects at each time. T-bars represent SE of the mean. Serum GM-CSF was undetectable in six healthy individuals at all (similar) times after receiving a single administration of inhaled placebo (not shown). The corresponding numeric raw data (mean, SD, (n)) are included elsewhere (online supplemental table S8). GM-CSF, granulocyte-macrophage colony stimulating factor.

S9). The calculated maximum concentrations were 9.1, 40.7 and 34.1 pg/mL, respectively, after administration of 150 µg, 300 µg and 600 µg of inhaled molgramostim, indicating 'more-than-proportional' changes at the lower doses and 'less-than-proportional' changes between higher doses (figure 1, online supplemental table S8). Similarly, $AUC_{0\text{-last}}$ increased by 6.8-fold and 1.6-fold, and $AUC_{0\text{-inf}}$ increased by 1.4-fold and 1.8-fold, respectively, for the twofold increase in dose (150 µg to 300 µg doses and 300 µg to 600 µg doses) (online supplemental table S8). The serum rGM-CSF half-life was <2 hours for the 150 µg and 300 µg doses and 5.9 hours for the 600 µg dose. The total body clearance at steady state (CL_{ss}/F) for which there were multiple determinations was 2260 L/h to 2850 L/h for the 300 µg dose and the 600 µg dose, respectively.

MAD Study

Endogenous GM-CSF in serum was below the LLoQ in all subjects before administration on Days 1, 4, 5 and 6 and at all times in subjects receiving placebo (online supplemental table S10). After inhaled molgramostim administration, serum rGM-CSF was detected by 0.5 hours with a T_{max} at 2 hours on Days 1 and 6 (figure 2, online supplemental tables S10 and S11). A twofold higher dose (300 µg vs 600 µg) resulted in a threefold higher C_{max} (32.1 vs 96.4 pg/mL) on Day 1 indicating 'more-than-proportional' PK (online supplemental table S10).

A similar trend was observed for measures of systemic exposure to rGM-CSF, $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$, which increased by 3.6-fold and 2.5-fold, respectively, for the same twofold increase in dose (online supplemental table 10). The same twofold higher dose followed similar PK on Day 6 with a 2.8-, 3.2- and 2.9-fold increase in C_{max}, $AUC_{0\text{-last}}$ and $AUC_{tau,}$ respectively. These results differ from those of the SAD study, which had a smaller sample size than the MAD study (n=4 vs n=9, respectively). At the 300 µg dose, serum rGM-CSF remained above the LLoQ in all subjects (n=9) through 6 hours on Day 1 and through 8 hours on Day 6 (online supplemental table S10). At the 600 µg dose, serum rGM-CSF levels remained above the LLoQ in most (7 of 9) subjects through 12 hours on Day 1 and 8 hours on Day 6. Serum GM-CSF fell below the LLoQ by 12 hours in most (7 of

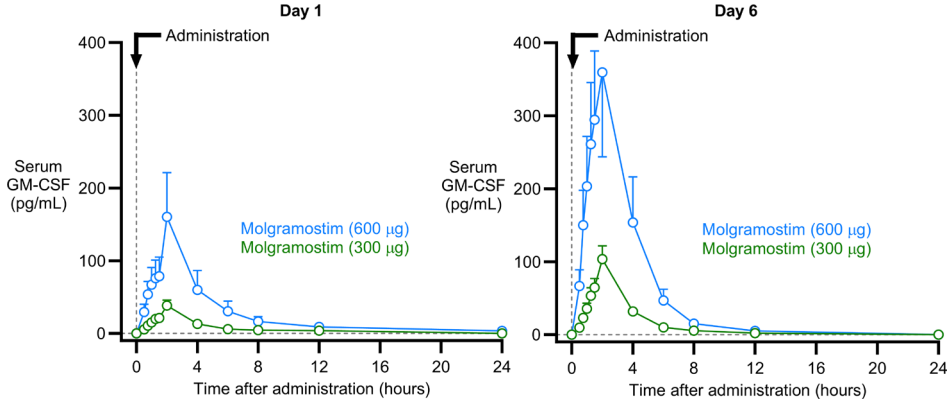

Figure 2 Serum GM-CSF in the multiple ascending dose study. 18 (n=9 per dose) healthy individuals received six once-daily administrations of inhaled molgramostim at 24-hour intervals on study days 1–6 and serum GM-CSF was measured at the indicated times (–0.25, 0.5, 0.75, 1, 1.25, 1.50, 2, 4, 6, 8, 12, and 24 hours on days 1 and 6, and at –0.25 hour on days 4 and 5). Data obtained on day 1 and 6 are shown in the left and right panels, respectively. Serum GM-CSF was undetectable in all subjects before dosing on study Days 1, 4, 5 and 6. Symbols represent the mean value among nine healthy subjects at each time. T-bars represent SE of the mean. Serum GM-CSF was undetectable in nine healthy individuals at all (similar) times after receiving a single administration of inhaled placebo (not shown). The corresponding numeric raw data (mean, SD, (n)) are included elsewhere (online supplemental table S10). GM-CSF, granulocyte-macrophage colony stimulating factor.

9) subjects and by 24 hours in all subjects (n=9) at each dose (online supplemental table S11). The serum rGM-CSF half-life ($t_{1/2}$) was similar (4.0 vs 3.7 hours, respectively) at both molgramostim doses (online supplemental table S10). Total body rGM-CSF clearance (CL_{ss}/F, calculated as dose/$AUC_{0\text{-inf}}$) was similar at both doses on Day 1 (2330±1550 vs 2110±1400 L/hr for 300 µg and 600 µg doses, respectively; n=6, 9; p=0.809) and on Day 6 (1180±615 vs 1030±847 L/hr for 300 µg and 600 µg doses, respectively; n=7, 9; p=0.700) (online supplemental table S10). Despite the short half-life, serum rGM-CSF appeared to accumulate following repeated administration of either dose as demonstrated by the AUC accumulation ratio (RAUC) and C_{max} accumulation ratio (RC_{max}). The Swing was not different for the two doses (p=0.053), suggesting sampling variability was similar (online supplemental table S11). The linearity index (LI) for the two doses was numerically, but not significantly, different (2.7±2.7 vs 2.5±1.4, n=6, 9, respectively; p=0.87) suggesting single-dose PK parameters may not predict serum rGM-CSF PK following multiple administrations (online supplemental table S10).

PD of Inhaled Molgramostim

Peripheral Leucocyte Counts

Inhaled molgramostim was associated with a transient increase in WBCs 6 hours following administration of a single dose of 150 µg, 300 µg and 600 µg (p=0.031, 0.043 and 0.177, respectively, for comparisons to placebo), although the latter was not statistically significant and the increases were not proportional to the dose; all values normalised and were similar to placebo (p>0.11) at Days 8 and 15 (figure 3A, online supplemental table S12). This same pattern was reflected by the molgramostim-stimulated increase in neutrophils and monocytes at 6 hours and increase in eosinophils at 24 hours (figure 3A, online supplemental table S12). Lymphocyte counts were decreased at 6 hours similarly for 300 µg and 600 µg doses and returned to baseline (similar to placebo) by 8 days (figure 3A, online supplemental table S12).

Repeated, once-daily inhaled molgramostim administration resulted in a characteristic sawtooth pattern of increased WBCs with daily peaks and troughs that increased in dose-dependent fashion until plateauing on Day 3 and returning to baseline (similar to placebo) by 21 days (figure 3B). The numbers of neutrophils, monocytes and eosinophils followed a similar pattern, except that eosinophil counts appeared to plateau later (figure 3B). Lymphocyte counts were decreased at 6 hours similarly for 300 µg and 600 µg doses and returned to baseline (similar to placebo) by 6 days (figure 3B, online supplemental tables S13-S17).

Fractional Exhaled Nitric Oxide

The FeNO concentration was not increased over the 24-hour observation period after a single administration of inhaled molgramostim at doses of 150 µg or 300 µg but was numerically, but not significantly, increased at the 600 µg dose (figure 4A, online supplemental table S18). Similarly, six daily administrations of inhaled molgramostim at 300 µg resulted in FeNO levels similar to placebo at all time points while the 600 µg dose resulted in numerical (but not statistically significant) increases at all time points after 24 hours on Days 1–6 (figure 4B, online supplemental table S19). Notwithstanding these minor changes, most values were below the cut-off used to identify eosinophilic airway inflammation,[34] and all were less than the upper limit of normal range for people without asthma or atopy (figure 4).[35]

DISCUSSION

This phase 1 clinical trial evaluated the safety, PK, and PD of inhaled molgramostim in healthy adults. Results showed that inhaled molgramostim was safe and well tolerated in healthy people with only expected pharmacological effects. Given that many PK parameters—such as C_{max}, AUC and half-life—may be unfamiliar to clinicians without a pharmacology background, we have provided a brief overview of their significance. Cmax reflects the peak systemic exposure following inhalation, while AUC represents the overall exposure over time and is particularly relevant for assessing dose proportionality and systemic absorption. The elimination half-life ($t_{½}$) indicates how long the drug remains measurable in circulation and helps inform dosing frequency. These parameters were derived using standardised non-compartmental methods aligned with regulatory guidelines (FDA, EMA and ICH) and calculated via Phoenix WinNonlin, a validated tool commonly used in early-phase clinical research. This approach ensures methodological consistency and allows for cross-study comparison, while also facilitating interpretation for respiratory physicians and other clinicians. PK parameters were determined in sequential SAD and MAD studies, the anticipated PD effects – transiently increased leucocyte total and subpopulation counts – were observed, and FeNO was found to be within the normal range at all doses tested. Molgramostim was well tolerated with no SAEs, dose-limiting toxicities or identified safety concerns.

In healthy people, molgramostim was rapidly absorbed after inhalation and resulted in picogram serum levels of rGM-CSF, a C_{max} at 2 hours and a half-life of less than 6 hours at all doses. While rGM-CSF was readily detectable in serum of healthy people after single or repeated administration(s) of molgramostim at a dose of 300 µg (C_{max}, 40.7 or 90.0 pg/mL, respectively), neither endogenous serum GM-CSF nor exogenously administered rGM-CSF was detectable in 137 of 138 aPAP patients after daily administration of inhaled molgramostim at a similar dose for 24 weeks.[29] Additionally, systemic exposure after inhaled administration appears to be far less than after intravenous or subcutaneous administration since one 300 µg dose of inhaled molgramostim resulted in a C_{max} of 32.1 pg/mL (this study) while one 250 µg dose

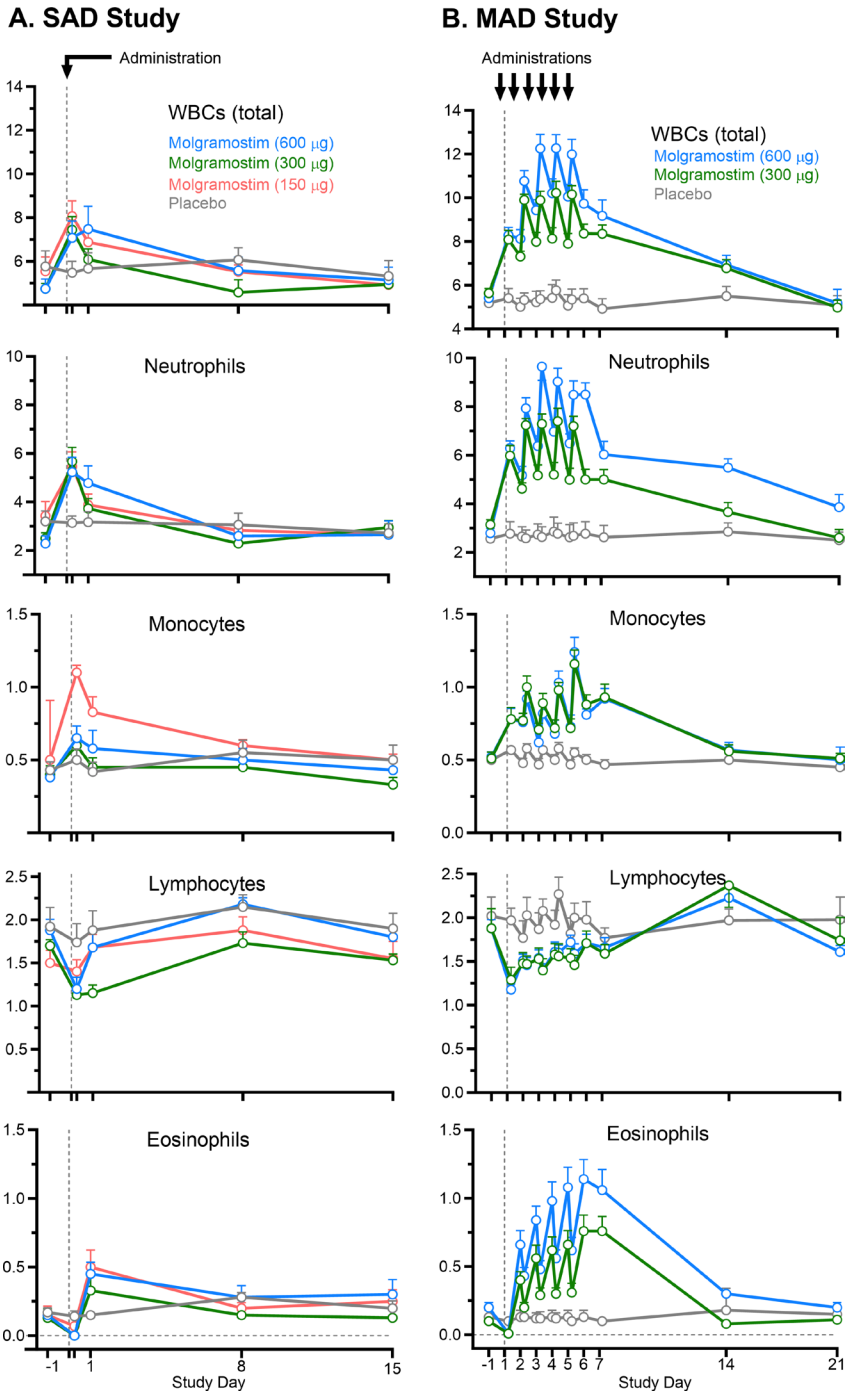

Figure 3 Effects of inhaled molgramostim on numbers of circulating white blood cells (WBCs). **A**. Single ascending dose study. Healthy individuals received inhaled molgramostim as described in the legend to figure 1 and the numbers of WBCs and WBC subsets (indicated) were determined at the indicated times (–0.25 and 6 hours, and 1, 8±2, and 15±2 days). Symbols represent the mean value among healthy subjects (four for each molgramostim dose, 6 for placebo). T-bars represent SE of the mean. The corresponding numeric data (mean, SD, (n)) are included elsewhere (online supplemental table S12). **B**. Multiple ascending dose study. Healthy individuals received inhaled molgramostim as described in the legend to figure 2 and the numbers of WBCs and WBC subsets (indicated) were determined at the indicated times (–0.25 and 6 hours on days 1 to 6, and –0.25 hours on days 7, 14±2, and 21±2 days). Symbols represent the mean value among healthy subjects (nine for each molgramostim dose, 6 for placebo). T-bars represent SE of the mean. The corresponding numeric raw data (mean, SD, (n)) are included elsewhere (online supplemental tables S13-S17).

of sargramostim (rGM-CSF produced in yeast) resulted in C_{max} of 1500 pg/mL or 5400 pg/mL after subcutaneous or intravenous administration,[31] which are 47-fold and 156-fold higher, respectively. These results underscore the negligible systemic exposure to rGM-CSF expected after administration of inhaled molgramostim in aPAP

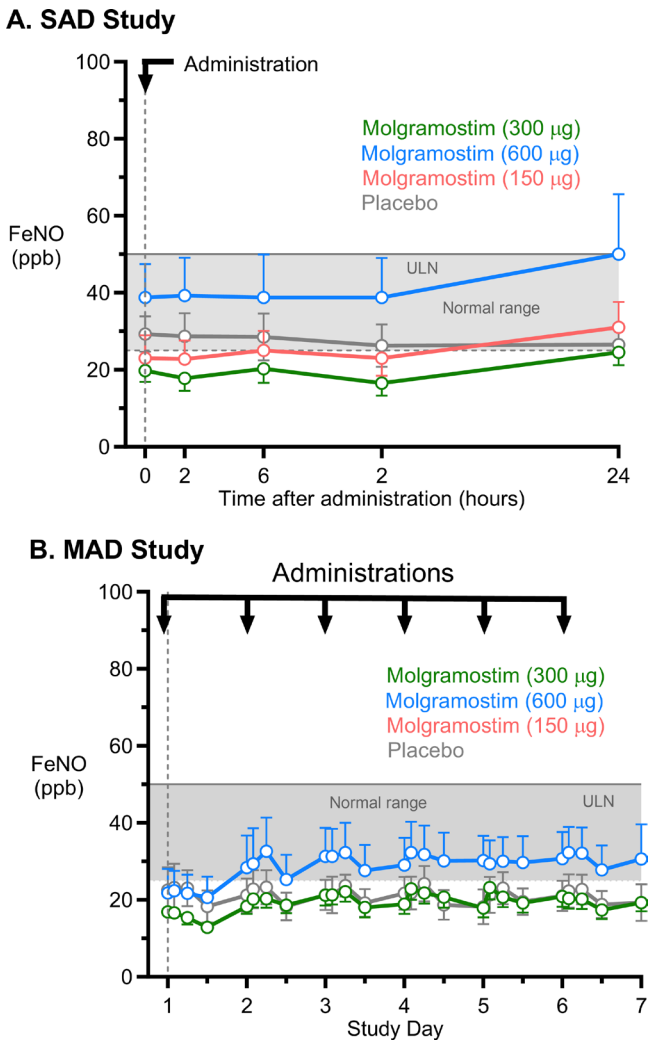

Figure 4 Fractional exhaled nitric oxide (FeNO) concentration following inhaled molgramostim administration. Healthy individuals received inhaled molgramostim as described in the legend of figure 1 (A) or figure 2 (B) and the FeNO concentration in breath condensate (expressed as parts per billion, ppb) was measured at the indicated times before (-0.5 to −0.25 hours) and 2, 6, 12 and 24 hours after each administration. Symbols represent the mean value among healthy subjects (A): four for each molgramostim dose and six for placebo; (B): nine for each molgramostim dose and six for placebo). T-bars represent SE of the mean. The range of FeNO in individuals without airway inflammation is 25 ppb to 50 ppb (grey box).[35] The corresponding numeric raw data (mean, SD, (n)) are included elsewhere (online supplemental table S18 and S19). FeNO, fractional exhaled nitric oxide; MAD, multiple ascending dose; SAD, single ascending dose; ULN, upper limit of normal.

patients. As sargramostim was developed for intravenous administration, its pharmacology after inhaled administration was not studied initially. However, recent interest in aerosol administration led to a small PK study in healthy people in which each of three participants received one 250-µg dose of inhaled sargramostim using a Pari LC-Star jet nebuliser (the dose and delivery method used in a recent clinical trial,[28] which resulted in a mean C_{max} of 9.1±4.2 pg/mL.[36] In the present study, one administration of a roughly similar dose of molgramostim (300 µg) delivered with an efficient, ultrasonic vibrating mesh nebuliser (eFlow, PARI Pharma) resulted in a mean C_{max} of 40.7 pg/mL.

Our observation of non-linear PK for rGM-CSF in serum after administration by aerosol inhalation at low and high molgramostim dose ranges is an interesting finding for which a molecular explanation is suggested by the detection of GM-CSF autoantibodies in healthy individuals,[37] although at low levels (1.0 (0.6–1.7) µg/mL, n=72) well below the threshold level (5 µg/mL) associated with an increased risk of developing aPAP.[38] For a 70 kg healthy adult, the typical blood volume (5000 mL) would be expected to contain an estimated 5200 µg of GM-CSF autoantibody, which would be available to bind exogenously administered rGM-CSF and render it undetectable by the GM-CSF antigen-capture ELISA used here. Based on a molar binding ratio of 7.8 to 1[16] and a molecular mass ratio of 10 to 1 for GM-CSF autoantibodies and GM-CSF, respectively, this quantity of circulating GM-CSF autoantibodies could bind an estimated 67 µg of the exogenously administered rGM-CSF.[38] Thus, rGM-CSF passing into the blood compartment in a healthy adult could become bound to circulating GM-CSF autoantibodies and a portion (up to 67 µg) rendered undetectable by a GM-CSF capture ELISA. Consequently, in the lower dose range (150 µg – 300 µg), less-than-proportional serum PK would be expected and at higher doses (300 µg to 600 µg), more-than-proportional PK would be expected.

Our observation that molgramostim caused a rapid, transient increase in leucocyte and leucocyte subpopulation counts is consistent with our observation that rGM-CSF was detected in blood following aerosol inhalation and with prior reports demonstrating systemic exposure to rGM-CSF causes these PD effects in healthy people.[9 10] In contrast, similar effects are not observed after systemic rGM-CSF exposure in aPAP patients, presumably due to the presence of high concentrations of GM-CSF autoantibodies in the blood and tissues,[10] which are capable of neutralising rGM-CSF at levels far higher (~50 000 fold) than are present physiologically.[16]

Our observation that inhaled molgramostim was safe and well tolerated in healthy people is an important finding. Its use as therapy for aPAP is also supported by the absence of detectable serum GM-CSF and absence of changes in blood leukocytes or GM-CSF autoantibody levels observed in three large studies evaluating molgramostim (IMPALA and IMPALA-2 trials)[29 39] or sargramostim (PAGE trial).[28] The observation that FeNO was similar in placebo-treated and molgramostim-treated subjects at doses of 150 µg and 300 µg and only mildly increased at a dose of 600 µg, which were all within the range reported for healthy people without asthma or atopy,[35] suggests clinically significant pulmonary eosinophilia is not present after inhalation of molgramostim at doses <300 µg but may be present at a dose of 600 µg.

Study limitations include the small sample size, which was appropriate for a first-in-human phase 1 study and mitigated by larger data sets available from the IMPALA[29] and IMPALA-2[39] trials. A second limitation was the use of an ELISA with a LLoQ of 2 pg/mL for measurement of GM-CSF in serum. The ELISA was not able to detect endogenous GM-CSF at the low levels of GM-CSF present physiologically, but which are functionally important.[13 16 37] Notwithstanding, the assay readily detected exogenously administered rGM-CSF at all doses tested. Further, the LLoQ corresponded to a small portion (2.2%) of the C_{max} observed after repeated administrations at the target dose (300 µg), results that are particularly relevant to the treatment of aPAP patients under current clinical testing.[29]

Given that no pharmacologic agents have received regulatory approval for use as therapy for aPAP in the United States or Europe, this study is an important step toward our understanding of the use of inhaled rGM-CSF as therapy of aPAP. The current standard-of-care for aPAP is whole lung lavage, which is invasive, resource intensive, carries defined procedural risks, does not address the underlying cause of disease and must be repeated regularly. Hence, there is a need for a pharmacotherapy that is well-tolerated, efficacious and addresses the underlying pathophysiology of the disease. While the safety and efficacy of molgramostim inhalation solution is currently being evaluated in patients with aPAP in the IMPALA-2 clinical trial (NCT04544293),[39] this study showed that inhaled molgramostim is well-tolerated, is absorbed systemically at picogram levels and shows an effect on WBC differentials mostly within normal reference ranges.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by The protocol, protocol amendments, and information provided to subjects were reviewed and approved by the Office for Research Ethics Committees Northern Ireland (ORECNI; approval no. 15/NI/0037). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request. Savara Inc. will consider requests for disclosure of clinical study participant-level data from qualified academic investigators. Recipients must provide evidence that participant privacy is assured through methods such as data de-identification, pseudonymisation, or anonymisation (as required by applicable law), and if such disclosure was included in the relevant study informed consent form or similar documentation. Qualified academic investigators may request participant-level clinical data and supporting documents (statistical analysis plan and protocol) pertaining to studies sponsored by Savara Inc. at medicalinfo@savarapharma.com.

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