Published in final edited form as:
J Clin Pharmacol. 2014 March ; 54(3): 341–350. doi:10.1002/jcph.203.

A FIM Study to Assess Safety and Exposure of Inhaled Single Doses of AP301—A Specific ENaC Channel Activator for the Treatment of Acute Lung Injury

Richard Schwameis, MD[1], **Sandra Eder, MD**[1], **Helmut Pietschmann, MD**[2], **Bernhard Fischer, PhD**[2], **Hermann Mascher, PhD**[3], **Susan Tzotzos, PhD**[2], **Hendrik Fischer, PhD**[2], **Rudolf Lucas, PhD**[4], **Markus Zeitlinger, MD**[1,a], and **Robert Hermann, MD, PhD**[5,b]

[1]Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria

[2]APEPTICO GmbH, Vienna, Austria

[3]Pharm-Analyt Labor GmbH, 2500 Baden, Vienna, Austria

[4]Department of Pharmacology and Toxicology, Vascular Biology Center, Georgia Health Sciences University, Augusta, GA, USA

[5]Clinical Research Appliance, Heinrich-Vingerhut-Weg 3, D-63571, Gelnhausen, Germany

Abstract

AP301 is an activator of ENaC-mediated Na^+ uptake for the treatment of pulmonary permeability edema in acute respiratory distress syndrome (ARDS). The purpose of this “first-in-man” study was to examine local and systemic safety and systemic exposure of ascending single doses of AP301, when inhaled by healthy male subjects. In a double-blind, placebo-controlled study, 48 healthy male subjects were randomized to 6 ascending dose groups (single doses up to 120 mg) of 8 subjects each (3:1 randomization of AP301: placebo). Serial assessments included spirometry, exhaled nitric oxide (eNO), vital signs, ECG, safety laboratory, adverse events (AE), and blood samples for the quantification of AP301 in plasma. Descriptive statistics was applied. All 48 subjects received treatment, and completed the study as per protocol. No serious, local (e.g.,

Corresponding Author: Markus Zeitlinger, Department of Clinical Pharmacology, Medical, University of Vienna, Waehringer Guertel 18–20, Vienna, Austria, markus.zeitlinger@meduniwien.ac.at.
[a]Principal Investigator.
[b]Fellow of the American College of Clinical Pharmacology (FCP).
Richard Schwameis and Sandra Eder contributed equally to the work.

Declaration of Conflicting Interests
Research funding for design and conduct of this study, collection, management, analysis, and interpretation of the data was sponsored by APEPTICO GmbH. H.P., B.F., S.T., H.F. are employees of APEPTICO GmbH. R.H. has been engaged by APEPTICO GmbH as clinical expert advisor for key aspects of the study design, conduct, and analysis, and has received financial support for research and consulting services from APEPTICO GmbH. H.M. has been contracted by APEPTICO GmbH as bioanalytical expert advisor and service provider for the bioanalytics of AP301 and has received financial support for these services. S.E., R.S., M.Z. are employees at the Medical University of Vienna which received grant support for the conduct of the study from APEPTICO GmbH. All authors contributed significantly to the overall planning of the study; analysis and interpretation of data; were involved in drafting the manuscript or revising it critically for important intellectual content; and gave final approval of the version to be published. R.L. received funds from the NIH/NHLBI grant RO1HL094609 for the preclinical research leading to this study.

Supporting Information
Additional supporting information may be found in the online version of this article at the publisher’s web-site.

hoarseness, cough, bronchospasm), or dose-limiting AEs were noted. None of the assessments indicated notable dose or time-related alterations of safety outcomes. Observed AP301 systemic exposure levels were very low, with mean C_{max} values of <2.5 ng/mL in the highest dose groups. Inhaled AP301 single doses up to 120 mg were safe and well tolerated by healthy male subjects. Distribution of inhaled AP301 was largely confined to the lung, as indicated by very low AP301 systemic exposure levels.

Keywords

AP301; ENaC activator; first-in-man study; ARDS

The diagnosis acute respiratory distress syndrome (ARDS) describes a type of acute diffuse, inflammatory lung injury, leading to increased pulmonary vascular permeability, pulmonary edema, increased lung weight, and loss of aerated lung tissue.[1,2]

ARDS represents a worldwide public health problem with high mortality, and specific pharmacological interventions targeting key pathophysiological processes of ARDS are still lacking.[3]

AP301 (scientific name: human tumor necrosis factor α [TNF-α]-derived peptide) is a synthetic peptide composed of 17 natural amino acids (Cys–Gly–Gln–Arg–Glu–Thr–Pro–Glu–Gly–Ala–Glu–Ala–Lys–Pro–Trp–Tyr–Cys), with a molecular mass of ~2,000 Da. Basically, AP301 is a circularized presentation of the lectin-like domain (so-called TIP domain) of human TNF-α.[4] Pulmonary administration of TIP peptide has been shown in a variety of small animal models of acute lung injury (ALI) to substantially alleviate pulmonary permeability edema of various pathophysiological conditions.[5–10] It was suggested, that either enhancement of alveolar fluid clearance (AFC) or improvement of reduced pulmonary vascular permeability (or both), could be the primary mode(s) of action, thereby leading secondary to remarkable improvements of impaired gas exchange.[10–13]

Recently, these data were confirmed by a large-animal study, in which acute lung injury (ALI) was induced by bronchoalveolar lavage followed by injurious ventilation in anaesthetized domestic pigs.[14] In this study, single administration of AP301 (i.e., nebulization of 1.0 mg/kg into the inspiratory branch of the ventilatory circuit), was associated with a significant, immediate and sustained decrease of lung fluid content, quantified by the extravascular lung water index (EVLWI). This effect was accompanied by a significant improvement of oxygenation (i.e., increase of PaO_2/FiO_2) and reduction of pulmonary shunt fraction (Q_s/Q_t). All these effects reflective of meaningful edema clearance, were maintained or gradually increased versus baseline and control group data over the entire observation period of 5 hours, thereby indicating a clinically useful effect duration of single oral AP301 inhalation.[14]

Recently, the primary pharmacology of AP301 was characterized by using chamber and whole cell patch clamp experiments in primary type II alveolar epithelial cells (AEC) isolated from rat, dog, and pig lungs.[15] In the presence of AP301, amiloride-sensitive Na^+ currents (via ENaC) in rat, dog, and pig AEC type II cells were increased by about 9-, 13-,

and 16-fold, respectively, versus baseline conditions.[15] These effects could be inhibited by the specific ENaC inhibitor amiloride. These results provide strong evidence that the pulmonary edema-clearing effect of AP301 is based on activation of the amiloride-sensitive Na^+ current through ENaC in type II AECs across all tested species. This implies that AP301 mediates its favorable effects predominantly by upregulation of vectorial Na^+ transport as driver of AFC. This is of importance, as available data suggest that ENaC function is reduced or down-regulated under the conditions of pulmonary permeability edema.[16]

As permeability edema is a frequent complication in a number of severe and life-threatening pulmonary conditions, such as ARDS, cardiogenic edema, high altitude pulmonary edema (HAPE), or ischemia-reperfusion injury, the latter of which can cause primary graft failure following lung transplantation, AP301 is a promising candidate with potential therapeutic value in all of these serious clinical pulmonary conditions.[11,12]

The aim of this paper is to present the overall translational concept and results of the first-in-man (FIM) study of AP301, which examined the local and systemic safety, and systemic exposure of ascending single doses of orally inhaled AP301 in healthy adult male subjects.

Methods

Ethical Conduct of the Study

The study protocol (EudraCT Number: 2011-000223-33), subject information and informed consent, and other pertinent information were reviewed and approved the Ethics Committee of the Medical University of Vienna and the Federal Health Authorities of Austria (AGES). The study was conducted in accordance with the principles of the International Conference on Harmonization guideline of Good Clinical Practice and the revised Declaration of Helsinki of the World Medical Assembly (Seoul, Korea, 2008). All subjects gave written informed consent, after having received ample information about the product characteristics, the study plan, and procedures, and the overall scope, meaning, and consequences of the trial.

Subjects

Male subjects of any ethnic origin underwent thorough evaluation of their medical and surgical history, full physical examination including vital signs, spirometry, exhaled nitric oxide (eNO), electrocardiogram, evaluation of biochemistry and hematology, hepatitis and HIV serology, illicit drug urine screen, and alcohol breath tests. Subjects were required to be healthy non-smokers, aged between 18 and 45 years (inclusive), having a body weight (BW) between 60 and 85 kg (inclusive), and a body mass index (BMI) between 20 and 29 kg/m^2 (inclusive). Exclusion criteria for study participation are detailed in the Online Supplement. If deemed suitable, subjects were enrolled and randomized into the study.

Study Design and Operational Conduct

This FIM study has been designed in agreement with the EMA “Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal

products,"[17] and with the FDA Guidance "Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers."[18]

The study followed a randomized, double blind, placebo controlled, parallel group, sequential ascending single-dose design. A total of 48 healthy adult male subjects were to be enrolled and randomized to 6 ascending dose groups of 8 subjects each. In each dose group, 6 subjects were randomly assigned to receive AP301, that is, orally delivered AP301 single doses of 4.32 (D1), 12 (D2), 30 (D3), 60 (D4), 90 (D5), or 120 mg (D6), and 2 subjects received matching placebo. This implies that the incremental dose factors were designed to gradually decrease with increasing AP301 dose levels, ranging from about 2.8 (i.e., 2.8-fold increment from D1 to D2) to 0.3 (i.e., 30% increment from D5 to D6). Details on incremental dose factors that were applied at each dose level are summarized in Table 1.

For safety reasons, the sequence and timing of AP301 administration within each dose group was so scheduled that throughout the trial in no instance more than two subjects were allowed to receive treatment on a given day. In such cases, that is, when more than one subject was scheduled for treatment on a given day, the minimum time interval to be observed between administrations of two subjects was 1.5 hours, without notable worrisome adverse safety observations in the subject dosed first.

Between dose groups, a minimum interval of one week was observed from the conclusion of clinical assessments of the preceding dose group before embarking on the next dose increment, in order to allow a Safety Data Review Board to carefully examine the safety, tolerability and exposure results of the preceding dose group, and to confirm that no stopping rules could be identified at subject or cohort level, and that the study could proceed according to protocol. No new treatment group was started without unanimous agreement and approval by the Safety Data Review Board and the Principal Investigator.

Subjects were hospitalized from Day 1 until 24 hours post-dose. During confinement they received standardized meals and beverages. A final end-of-study visit was conducted 7–10 days post-dose.

Investigational Medicinal Product

AP301 was provided as sterile lyophilized powder for solution in glass vials containing 25 mg (Aptuit Ltd., Glasgow, UK), to be reconstituted in 1.0mL water for injection prior to nebulization for oral inhalation, yielding a final concentration of AP301 in the obtained solution of 25 mg/mL. As matching placebo, sodium chloride 0.9% solution was used (10mL ampoules; B. Braun Melsungen AG, Germany), because it was shown to compare very closely to reconstituted AP301 in terms of water-clear appearance, taste, and nebulization rate, conditions that were considered to be prerequisites for maintaining double blinding. The pH of the AP301 25 mg/mL solution was determined with 4.40, the osmolarity with 23mosmol/L, and the viscosity was found to be only marginally different from water (1.07 mPa s). The theoretical osmolarity of sodium chloride 0.9% solution amounts to 309mosmol/L, and the pH may range between 4.5 and 7.0. Hence, both formulations differed only in terms of osmolarity to some extent.

Aerosol Generation and Delivery

Based on prestudy aerosol characterization, the Aeroneb® Solo single patient use vibrating mesh liquid nebulizer, powered by the Aeroneb® Pro-X Controller (Aerogen, Galway, Ireland), was selected for delivery of both the AP301 and the placebo (sodium chloride 0.9%) aerosols. The nebulizer system was shown to produce a continuous fine particle, low velocity aerosol, optimized for targeted drug delivery to the smallest airways and deepest parts of the lungs.

To enable a double-blind study performance, study medications were reconstituted, and nebulizers labeled and prefilled according to the randomized dosing schedule by Hospital Pharmacy staff. As the maximum medication cup capacity of the nebulizer is 6.0 mL, two nebulizers were provided for the administration of the two highest dose levels (i.e., doses D5 and D6).

All drug inhalations were performed in the mornings of study days at 8.00 a.m. ± 1 hour. Subjects were instructed to inhale the AP301/placebo aerosols by steady tidal volume breathing in seated position, by using a mouthpiece (Aeroneb® single use, 22 mm) and wearing a nose clip. Subjects were supervised by clinical staff for regular breathing maneuvers and possible occurrences of adverse events (AEs) throughout the entire inhalation procedure. Inhalation times ranged from 1–2minutes (D1) to about 30minutes (D6, mean 31.38 ± 4.9 min). The conclusion of inhalation of the complete dose volume was defined as time zero (t_0) for all subsequent study assessments and activities.

Aerosol Characterization

Particle distribution of the AP301 aerosol generated by the Aeroneb® Solo nebulizer was determined by using a Next Generation Impactor (NGI) at 15 L/min, and laser diffraction (HELOS laser diffractometer; Sympatec GmbH, Clausthal-Zellerfeld, Germany). By laser diffraction, the mean fine particle fraction (FPF) was determined to be about 60% for the 25mg/mL solution, and the average droplet size to be 3.3 μm. Breath simulator tests indicated that approximately 70% of the metered dose was collected on the inspiration filter, representing the estimate of the delivered AP301 dose ex mouthpiece in the clinical setting (Apeptico Research Report, data on file).

Considering these data together with the FPF of 60% as determined by NGI, it was estimated that about 42% of the nebulizer filling dose (i.e., metered dose) or about 60% of the orally delivered dose (ex nebulizer mouthpiece) would be deposited in the lungs of human subjects (i.e., respirable dose). This estimate of the respirable dose also provided the basis for the calculation of the expected dose-related total human lung exposure, and the determination of a safe AP301 starting dose based on available animal data, although for safety reasons a 100% lung deposition of the orally delivered AP301 dose (ex nebulizer mouthpiece) was assumed (see also below). Throughout the manuscript, however, the dose referred to is always delivered AP301 doses ex nebulizer mouthpiece and nebulizer filling doses, unless stated otherwise. A summary of the dosing information by dose group is provided in Table 1.

Calculation of Human Starting Dose

The AP301 human starting dose was calculated with reference to the FDA Guidance "Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers,"[18] and under careful consideration of specific additional requirements dictated by the orally inhaled route of administration and the highly consistent observation from preclinical trials in mice, rats, and dogs that AP301 was hardly absorbed from the pulmonary compartment into the systemic circulation, that is, AP301 distribution was largely confined to the lungs across all species tested [APEPTICO, data on file]. For this reason human equivalent lung doses (HELDs) were calculated and scaled based on species specific lung weight factors (LWFs) and approximation of total lung exposure (i.e., dose/kg lung weight) achieved in 14-day inhalation toxicology studies in rats and dogs, rather than calculation of standard human equivalent doses (HEDs), based on BW or body surface area (BSA). The approximation of species-specific lung doses included the consideration of the aerosol characteristics applied in the preclinical studies (i.e., approx. 60% fine particles; mass median aerodynamic diameter (MMAD) of ≤3.0 μm), whereby the dose fraction of particles of MMAD ≤3.0 μm was considered to become deposited in the lung/airways, that is, to represent the actual lung dose.[19,20]

Because the lowest "no observed adverse effect level" (NOAEL) was observed in the dog, and for other reasons, such as the high sequence identity between dog and human ENaC proteins (87.0%), and similarities in respiratory tract anatomy and respiratory/breathing physiology and pattern, the dog was considered the most relevant (i.e., human like) species for the prediction of AP301-mediated respiratory tract effects in humans. Based on the NOAEL data obtained in the dog, along with the approximated total lung exposure achieved in this species, the scaled HELD, and an additional safety factor of 50 applied to the data, together with the conservative assumption that 100% of the delivered dose (ex inhalator mouthpiece) would be deposited in human lungs, a maximum recommended starting dose (MRSD) of AP301 of 0.1 mg/kg BW (nebulizer filling dose) was determined.

Another approach of calculating the HELDand thus the starting dose uses the minimal anticipated biological effect level (MABEL). The MABEL was approximated with 0.5 mg AP301/kg BW from pharmacological in vivo animal studies. Similar to the NOAEL approach the HELD was scaled and calculated based on species specific LWFs.

As the NOAEL approach provided the lower estimate of the human starting dose, and was considered most robust as it was derived from 2-week toxicology studies, the NOAEL approach was applied in the present study for the starting dose calculation.

Based on the known tissue expression pattern and the physiological role of the target (ENaC), and the pharmacological characteristics of AP301 along with its expected low systemic exposure levels, AP301 was not considered to be a "high risk" new biological entity according to the criteria outlined in the EMA "Guideline on strategies to identify and mitigate risks for first-inhuman clinical trials with investigational medicinal products."[17]

Safety and Tolerability Assessments

Serial safety assessments such as spirometry, exhaled nitric oxide (eNO), systolic/diastolic blood pressure (SBP/DBP), pulse rate, ECG, safety laboratory, and the subjects' well being in terms of nature, severity and incidence of AEs were collected over 24 hours post-dose. All safety measurements were done according established standard methodologies. Lung function assessments (FEV_1 and FVC) and quantification of eNO were performed in compliance with the ATS/ERS standards,[21] and QT interval data (QT/QTc) of the ECG were analyzed in keeping with the recommendations provided by the ICH E14 guideline.[22] Methodological details are provided in the Online Supplement.

Blood and Urine Sampling, Sample Processing, and Bioanalytical Methods

Serial blood samples for pharmacokinetic analysis of AP301 in plasma were collected pre-dose, at the end of AP301 inhalation (t_0), 5, 10, 20, 40, 60, and 120 min after complete inhalation of study medication. Urine was collected after subjects had emptied their bladder prior to AP301 administration over a 4-hour interval post-dose. Methodological details of the sample processing are summarized in the Online Supplement.

Plasma and urine concentrations of AP301 were quantified using a validated internally standardized liquid chromatography tandem mass spectrometry (LC-MS/MS) method with a lower limit of quantification (LOQ) of 1.0 ng/mL (plasma) and 1.0 ng/mL (urine), respectively. The inter-batch precision (CV) of the quality control samples in human plasma (conc. 2.50/20/240 ng/mL) ranged from 5.54% to 10.15%. The inter-batch accuracy (with reference to the mean value) of the quality control samples in plasma ranged from 96.1% to 99.9%.[23]

Pharmacokinetic Analyses

Drug concentration–time data for each subject were analyzed by standard non-compartmental pharmacokinetic methods. Methodological details are provided in the Online Supplement.

Sample Size Considerations and Statistical Analyses of Safety Data

As the overall objectives of "first-in-human" studies are— by definition—essentially exploratory, and comprise evaluation of multiple safety variables, no formal sample size and power calculations were employed. All clinical safety data were described using classic descriptive statistics, that is, mean, SD, CV(%), median, minimum and maximum values for quantitative variables and frequencies for qualitative variables, and examined for remarkable post-dose changes versus baseline and versus placebo at subject and cohort level.

Results

Subject Demographics and Disposition

A total of 63 subjects were screened, thereof 48 subjects were determined eligible for enrolment and randomized. The baseline demographic data of the study populations of all dose groups were adequately comparable; most of the enrolled subjects were of Caucasian origin, details are given in Table 2. All planned, 6 escalating dose groups (i.e., D1–D6) were

completed, and all 48 randomized subjects received their randomized treatment, completed the study according to protocol, and were included in the safety and PK analysis. Safety and Tolerability

Adverse events—All AP301/placebo inhalations were uneventful and regularly completed as scheduled. In particular no local adverse reactions such as hoarseness, local mucosal irritation, cough or signs and symptoms of bronchospasm were noted. No AE led to discontinuation of any subject from the complete administration of the randomized AP301/placebo treatment or to premature discontinuation of the study, and no serious AE (SAE) occurred throughout the study. Overall 13 treatment-emergent AEs (TEAEs) were noted, thereof 7 AEs were considered related to AP301 inhalation. All AEs were transient and of mild intensity and all of them resolved spontaneously, most (except one, which required paracetamol administration) without any therapeutic intervention.

In subjects randomized to AP301, AEs occurred at an overall frequency of 25% (i.e., 12/48 subjects), while the overall frequency of AEs in placebo-treated subjects was 42% (i.e., 5/12 subjects). The number of subjects reporting at least one treatment-related AE upon AP301 inhalation ranged between zero (D1, D6), one (D2, D4, D5), and two (D3), while one subject receiving placebo was considered to display a treatment related AE. Hence, neither a remarkable difference in the overall AE frequency between AP301 and placebo treated subjects was noted nor any dose relationship across the AP301 treatment groups.

The most frequently reported AE was nasopharyngitis with an overall frequency of 8.3% (4/48 subjects). The second most frequent AE was headache with an overall frequency of 4.2% (2/48 subjects). All other AEs occurred at an overall frequency of 2.1% (1/48 subjects).

Spirometry—Mean baseline values and post-dose time courses of FVC and FEV_1, the primary lung function outcome variables, are displayed by AP301 dose group and pooled placebo recipients in Figures 1 and 2, respectively. Because of the small sample size of dose groups, mean baseline lung function data were not perfectly homogeneous across groups and did show some scatter. However, there were neither at individual nor at dose group level remarkable declines of FVC or FEV_1 notable, indicating absence of relevant dose-dependent effects of AP301 on airway caliber or vital capacity. In particular, no FEV_1 declines of ≥20% from baseline were noted in any subject at any time point, and only a single subject receiving AP301 (D5) displayed a post-dose FEV_1 decline of ≥15% from baseline at various post-dose time points. In some of the dose groups, modest increases in FVC and FEV_1 outcomes over the first 6 hours post-dose were seen (Figures 1 and 2). Complete tabulated summaries of arithmetic mean (±SD) andmedian (ranges) FVC and FEV_1 data are provided in Online Supplement Tables S1 and S2, respectively.

Exhaled nitric oxide—Mean (±SD) baseline values and post-dose time courses of eNO are summarized in Online Supplement Table S3. Overall, inhalation of AP301 did not change the content of eNO at any dose level as compared to the respective baseline data and with corresponding mean and median values observed in the pooled placebo group. At an individual level 3 subjects displayed modest eNO elevations (1 placebo, 1 D2, and 1 D6

subject). However, the respective placebo and D6 recipient consistently showed modestly elevated eNO values between 44 and 74 ppb at all scheduled occasions including screening and baseline assessments, that is, no remarkable post-dose increases. Only one subject (D2) showed a modest post-dose increase from 33 ppb at baseline to 43 ppb at 6 h post-dose, which is within the established intra-subject variability of eNO assessments, and hence, not considered clinically meaningful.

Vital Signs

Mean baseline values and post-dose time courses of systolic (SBP) and diastolic (DBP) blood pressure are displayed by AP301 dose group and pooled placebo recipients in Online Supplement Figure S1, while mean (±SD) heart rate (HR) data (derived from ECG recordings) are summarized in Online Supplement Table S4. Although at dose group level there was some—probably procedure related—scatter in SBP and DBP over the first 2 hours post-dose notable, overall no clinically meaningful dose-or time-dependent effects of AP301 inhalation on vital signs were detected. This interpretation is supported by the stability of corresponding HR data (Online Supplement Table S4) and the absence of subject reported signs and symptoms suggestive of clinically meaningful hypotension.

Electrocardiogram—Mean baseline values and post-dose time courses of PQ and QTc intervals of the ECG are displayed by AP301 dose group and pooled placebo recipients in Online Supplement Figure S2. Overall, no clinically meaningful or remarkable dose-or time-dependent changes in any of the captured ECG outcome variables were noted. In particular, no QTc value exceeded the threshold (450 milliseconds) for potential concern for developing ventricular arrhythmias. Significant categorical QTc interval prolongations according to ICHE14,[21] as compared with mean baseline values were not observed at any dose level.

Safety laboratory—No clinically meaningful or remarkable dose-or time-dependent changes in any of the captured safety laboratory variables were noted, except some modest and transient isolated declines in leukocyte counts (below 4.0 G/L), which were observed at a frequency of 16.7–33% (i.e., 1/6 to 2/6 subjects) in the D3, D5, and D6 groups at 24 hours post-dose, without any accompanying changes in the respective differential white blood cell counts. Moreover, in 3/6 of these subjects lower leukocyte counts were already observed at screening or at baseline. The investigators considered all observations of low neutrophil counts as clinically non-relevant abnormalities, and all deviations normalized readily without intervention.

Pharmacokinetics

Mean (±SD) maximum (C_{max}) and total AP301 exposure ($AUC_{0–t}$) data as well as the median (ranges) times to achieve maximum plasma concentrations (t_{max}) are summarized in Table 3. As expected from preclinical data, systemic AP301 exposure was overall very low with observed maximum concentrations below LLOQ or <0.5 ng/mL at dose-level D1 (<LLOQ), and D2/D3 (<0.5 ng/mL), respectively. In dose groups D4, D5, and D6 maximum and total systemic exposure outcomes were well comparable, with mean C_{max} values ranging between 2.21 ± 1.75 ng/mL (D6) and 2.39 ± 1.75 ng/mL (D5), and AUC values

ranging between 45.52 ± 39.91 ng × min/mL (D5) and 55.80 ± 54.89 ng × min/mL (D4). The median t_{max} values observed ranged between 0 and 5 minutes post-dose (i.e., t_0) across dose groups, with no indication of any clear-cut dose dependency of this variable. AP301 could not be quantified in any of the urine samples of any subject.

Discussion

A key step in translational medicine is the progress from preclinical studies to the initial human trial, that is, the FIM study. Recent experience, including the unexpected near-fatal cytokine storm in the FIM study with the anti-CD28 superagonist TGN1412,[24] demonstrated limited ability to predict human effects, and that FIM studies may carry significant uncertainty.[25]

In FIM trials, one important safety aspect is the determination of an appropriate starting dose. The general approach is to calculate a safe starting dose based on the NOAEL from toxicology studies, that is, the highest dose level at which no biologically relevant adverse effects are observed in the most sensitive or human-relevant animal species.[18] The NOAEL dose is then scaled to a HED and finally appropriate safety factors are applied based on known product characteristics and the amount and importance of remaining uncertainties and safety concerns left by the existing preclinical knowledge base.[18]

The TGN1412 experience also pointed to the fact, that beside the NOAEL approach from toxicological studies, attention should be paid to the pharmacology of investigational drugs, that is, dose–response characteristics and minimum drug concentrations conferring biological effects, a concept that has been described as MABEL (i.e., *m*inimum *a*nticipated *b*iological *e*ffect *l*evel) approach. While there appears to be agreement that every effort should be taken to calculate the MABEL, there are often practical constraints in its accurate and reliable determination, and it should be born in mind that for certain drugs in vitro studies may substantially overestimate the MABEL in vivo.[26] However, application of both, NOAEL-and MABEL-based estimations of the human starting dose represents the current scientific “best practice” and is likely to provide the highest degree of confidence, in particular when both approaches deliver consistent estimates.

Additional challenges arise in the estimation of safety margins and starting doses with substances that are developed for administration by inhalation. For such products the reliable quantification (i.e., estimation) of the total lung dose achieved is a critical issue in preclinical as well as clinical studies. Actually, inhaled doses are subject to many anatomical, physiological, pharmaceutical and methodological factors (e.g., aerosol generation, etc.), and clearly more difficult to determine than parenteral or oral doses.[19] This creates additional challenges and uncertainties in the interspecies scaling of total lung doses. In the design and preparation of the present study, particular attention was paid to the requirement that comparable aerosol characteristics (i.e., MMAD ≤ 3.0 μm) were employed in the preclinical toxicology studies in rat and dog and the clinical FIM study, and the estimates of actual lung doses administered (i.e., fraction of dose with MMAD ≤ 3.0 μm),[19] while a highly conservative assumption of complete (i.e., 100%) lung deposition of the

delivered AP301 dose (ex inhalator mouthpiece) was applied to approximate the human lung dose in the FIM trial.

Because of the very limited and transient systemic exposure of AP301 consistently seen in all animal species, which in turn was consistent with the observation of hardly any systemic adverse effects in these studies, the achieved total lung dose in the most human-relevant species (i.e., dog) was considered the relevant key determinant for both the estimation of a safe starting dose and for the approximation of the maximum allowable human dose. However, technical/feasibility considerations, such as the 30 minutes time requirement for nebulization of a 120 mg AP301 dose, and in particular existing knowledge on the effective pharmacological AP301 dose range of 0.5–1.0 mg/kg BW in various animal models,[4,7,9,14] also co-determined the selection of the maximum targeted human dose.

As a consequence of the TGN1412 experience, the European Medicines Agency (EMA) has issued a guidance document, with the aim to support researchers and sponsors in the identification of risk factors, that is, the systematic assessment of the potential of investigational medicinal products to cause severe adverse reactions in the initial human use. Concerns may be derived from particular knowledge or lack thereof regarding the mode of action, the nature of the target, and/or the relevance of animal models, besides a variety of other product characteristics.[17]

The evaluation of respective AP301 risk characteristics indicated that neither the nature (biological role) nor the ENaC tissue expression pattern (i.e., AECs, kidney and gastrointestinal tract) would suggest any potential of AP301 to possibly confer effects on the human immune system, with the potential risk of displaying a “trigger pharmacology” toward exaggerated immune-cell stimulation and/or cytokine release.[26] Further, cell-based studies showed that ENaC activation by AP301 was clearly dose-dependent in the range of 5–100 nM. Also the high degree of sequence identity of ENaC across species and the demonstrated pharmacological activity of AP301 on primary type II AECs from dog, pig, and rat lungs,[9,15] provided reassurance that the safety findings from preclinical studies in rat and dog would adequately predict potential human risk.

Supportive evidence on the relative human safety of pharmacological alteration of alveolar ENaC activity was also available from the reported use of inhaled amiloride, an established ENaC inhibitor, in clinical studies involving adolescents and adults with cystic fibrosis,[27] and healthy adult subjects and patients with atopic asthma.[28] In these studies pharmacological inhibition of pulmonary ENaC by orally inhaled amiloride was generally well tolerated and did not result in altered airway caliber, bronchial (hyper-)reactivity or other clinically significant AEs. Hence, based on the available evidence, AP301 was not considered to be a “high risk” product according to the EMA guideline criteria.[17]

Besides the selection of a safe starting dose and the approximation of a maximum targeted dose, the design of a dose escalation strategy allowing for an adequate balance of safety, trial efficiency, and ethical conduct is the third critical aspect of FIM studies. In the present trial the incremental dose factors were designed to gradually decrease with increasing AP301 dose levels, ranging froma 2.8-fold dose increment from D1 to D2 to just under 30%

increment from D5 to D6 (for details see Table 1). This approach in principle followed a so-called modified Fibonacci sequence,[29] while not being exactly identical to it, and followed the main consideration that larger dose increments can be safely applied in the sub-pharmacological dose range, while smaller dose increments are advisable at the expected steep part of the dose–response curve. In terms of absolute dose increments, the designed dose escalation sequence resulted in fixed 30mg AP301 dose increments from dose-level D3 (30 mg) to D6 (120 mg). Thereby this approach limited the maximum allowable dose increment of AP301 to ≤0.5 mg/kg BW for each trial subject, which was considered to be an important safety feature of the dose-escalation strategy. In terms of trial efficiency, the employed dose escalation sequence enabled a total incremental dose factor of about 28-fold from D1 to D6, with just five incremental dose steps.

The AP301 exposure data obtained in the study are highly consistent with the expected limited systemic bioavailability of orally inhaled AP301, as suggested by the available preclinical evidence in various species [APEPTICO, data on file], as well as the molecular weight (~2,000 kDa) and overall physico-chemical properties of this cyclic peptide. Quantifiable maximum exposure values (C_{max}) exceeding 2.0 ng/mL at mean dose-cohort level were only observed from dose-level D4 (60 mg) onward. Interestingly, maximum and total (AUC) AP301 exposure remained constant with further dose increments (see Table 3), thereby suggesting either saturable/rate limited absorption processes or high clearance rates of systemically absorbed drug amounts, or a combination of both. The observation of readily observed median t_{max} values about 5 minutes post-dose (t_0), supports the contributing role of a high systemic clearance of AP301 in the observed limited and non-dose-proportional exposure characteristics of inhaled AP301.

The absence of a clinically meaningful systemic AP301 exposure across the investigated dose range is entirely consistent with the absence of any dose-related safety findings in clinical standard assessments of systemic safety such as vital signs (HR, SBP, DBP), ECG outcomes, safety laboratory variables, and AE reports.

Also the limited systemic availability of AP301 underpins the premise that upper and lower airways and the entire lung compartment are to be considered the organ systems of primary safety relevance for orally inhaled AP301. For these reasons attention was paid in our study to comprehensive safety assessments of lung function (FEV_1 and FVC), and the exploration of exhaled nitric oxide (eNO) levels as marker of potential inflammation-promoting effects of TNF-α derived AP301 on the airways, although it was realized that eNO cannot yet be considered as a validated marker of pro-inflammatory airway drug effects. Notably, none of these airway/lung safety assessments indicated any dose-related adverse effects in any of the outcome variables, at individual and dose group level. Thereby it is considered of particular importance that no FEV_1 declines of ≥20% from baseline were noted in any subject at any time-point, and that despite the multiple FEV_1 assessments with the inherent possibility of occasional “regression to the mean” events, only a single subject receiving AP301 (D5) displayed a post-dose FEV_1 decline of ≥ 15% from baseline. Overall these data indicate a lack of meaningful pharmacological AP301 effects on airway caliber. This outcome is consistent with absence of any signs and symptoms that would indicate a local irritating potential of AP301, such as local mucosal irritation, hoarseness, cough, or bronchospasm.

In conclusion, orally inhaled ascending single doses of AP301 up to 120mg were safe and shown to be well tolerated by healthy adult male subjects. Consistent with preclinical data in varies species, distribution of inhaled AP301 was largely confined to the lung, as indicated by very low maximum (C_{max}) and total (AUC_{0-t}) AP301 systemic exposure levels. None of the comprehensive safety assessments employed, incl. spirometry, eNO, SBP/DBP, HR, ECG, safety laboratory variables, or AE reports, indicated any clinically meaningful or remarkable dose-or time-related alterations of safety outcomes, neither at dose group nor at individual level. Overall, the data obtained in the study support a straightforward continuation of the clinical development of AP301 into Phase II clinical studies in patients affected by ARDS or developing ischemia-reperfusion injury in course of LTX surgery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant sponsor: APEPTICO GmbH, Vienna, Austria.

Funding

The study was sponsored by APEPTICO GmbH, Vienna, Austria.

References

1. Ranieri VM, Rubenfeld GD, et al. Definition Task Force ARDS. Acute respiratory distress syndrome: the Berlin definition. JAMA. 2012; 307:2526–2533. [PubMed: 22797452]
2. De Luca D, Piastra M, Chidini G, et al. The new “Berlin definition” of acute respiratory distress syndrome: clinical evaluation in infants and expert consensus. Intensive Care Med. 2013; 39(Suppl 1):S197–S198.
3. Phua J, Badia JR, Adhikari NKJ, Wendel A, Lucas R. Has mortality from acute respiratory distress syndrome decreased over time? Am J Respir Crit Care Med. 2009; 179:220–227. [PubMed: 19011152]
4. Lucas R, Magez S, De Leys R, et al. Mapping the lectin-like activity of tumor necrosis factor. Science. 1994; 263(5148):814–817. [PubMed: 8303299]
5. Braun C, Hamacher J, Morel DR, Wendel A, Lucas R. Dichotomal role of TNF in experimental pulmonary edema reabsorption. J Immunol. 2005; 175:3402–3408. [PubMed: 16116234]
6. Elia N, Tapponnier M, Matthay MA, et al. Functional identification of the alveolar edema reabsorption activity of murine tumor necrosis factor-alpha. Am J Respir Crit Care Med. 2003; 168:1043–1050. [PubMed: 12842853]
7. Vadasz I, Schermuly RT, Ghofrani HA, et al. The lectin-like domain of TNF-a improves alveolar fluid balance in injured isolated rabbit lungs. Crit Care Med. 2008; 36:1543–1550. [PubMed: 18434905]
8. Xiong C, Yang G, Kumar S, et al. The lectin-like domain of TNF protects from Listeriolysin-induced hyperpermeability in human pulmonary microvascular endothelial cells—a crucial role for Protein Kinase C-alpha inhibition. Vascul Pharmacol. 2010; 52(5–6):207–213. [PubMed: 20074664]
9. Hamacher J, Stammberger U, Roux J, et al. The lectin-like domain of TNF improves lung function after rat lung transplantation—potential role for a reduction in reactive oxygen species generation. Crit Care Med. 2010; 38(3):871–878. [PubMed: 20081530]

10. Lucas R, Yang G, Gorshkov B, et al. Protein kinase C-α and Arginase I mediate Pneumolysin-induced pulmonary endothelial hyperpermeability. Am J Resp Cell Mol Biol. 2012; 47(4):445–453.
11. Berthiaume Y. Tumor necrosis factor and lung edema clearance: the tip of the iceberg? Am J Respir Crit Care Med. 2003; 168(9):1022–1023. [PubMed: 14581284]
12. Matthay MA, Berthiaume Y. Novel molecular strategy to prevent pulmonary edema. Crit Care Med. 2008; 36:1671–1672. [PubMed: 18448936]
13. Mora-Esteves C, Dikdan G, Koneru B. Dr. Jekyll and Mr. Hyde saga of a cytokine: the devil in the details. Crit Care Med. 2010; 38(3):997–998. [PubMed: 20168164]
14. Hartmann EK, Boehme S, Duenges B, et al. An inhaled tumor necrosis factor-alpha-derived TIP peptide improves the pulmonary function in experimental lung injury. Acta Anaesthesiol Scand. 2013; 57:334–341. [PubMed: 23216436]
15. Tzotzos S, Fischer B, Fischer H, et al. AP301, a synthetic peptide mimicking the lectin-like domain of TNF, enhances amiloride-sensitive Naþ current in primary dog, pig and rat alveolar type II cells. Pulm Pharmacol Ther. 2013 Jan 9. pii: S1094–5539(13) 00003–5. Epub ahead of print. 10.1016/j.pupt.2012.12.011
16. Lee JW, Fang X, Dolganov G, et al. Acute lung injury edema fluid decreases net fluid transport across human alveolar epithelial type II cells. J Biol Chem. 2007; 282(33):24109–24119. [PubMed: 17580309]
17. EMA (CHMP). Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products. EMEA-CHMP/SWP/28367/07. Jul 19.2007
18. U.S. Department of Health and Human Services, Food and Drug Administration, Center for drug Evaluation and Research (CDER). Guidance for industry “estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers”. Jul. 2005 http://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf
19. Wolff RK, Dorato MA. Toxicologic testing of inhaled pharmaceutical aerosols. Crit Rev Toxicol. 1993; 23:343–369. [PubMed: 8155274]
20. Lewis TR, Morrow PE, McClellan RO, et al. Establishing aerosol exposure concentrations for inhalation toxicity studies. Toxicol Appl Pharmacol. 1989; 99:377–383. [PubMed: 2749728]
21. American Thoracic Society Documents. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005; 171:912–930. [PubMed: 15817806]
22. ICH. ICH harmonised tripartite guideline E14. The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. Oct. 2005 http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Guideline.pdf
23. Mascher D, Tscherwenka W, Mascher H, Fischer B. Sensitive determination of the peptide AP301—a motif of TNF-α—from human plasma using HPLC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci. 2012; 908:18–22.
24. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med. 2006; 355:1018–1028. [PubMed: 16908486]
25. Dresser R. First-in-human trial participants: not a vulnerable population, but vulnerable nonetheless. J Law Med Ethics. 2009; 37:38–50. [PubMed: 19245601]
26. Dayan CM, Wraith DC. Preparing for first-in-man studies: the challenges for translational immunology post-TGN1412. Clin Exp Immunol. 2008; 151:231–234. [PubMed: 18190459]
27. Jones KM, Liao E, Hohneker K, et al. Pharmacokinetics of amiloride after inhalation and oral administration in adolescents and adults with cystic fibrosis. Pharmacotherapy. 1997; 17:263–270. [PubMed: 9085317]
28. Knox AJ, Britton JR, Tattersfield AE. Effect of sodium-transport inhibitors on bronchial reactivity in vivo. Clin Sci (Lond). 1990; 79:325–330. [PubMed: 2171852]
29. Penel N, Kramar A. What does a modified-Fibonacci dose-escalation actually correspond to? BMC Med Res Methodol. 2012; 12:103–107. [PubMed: 22824322]

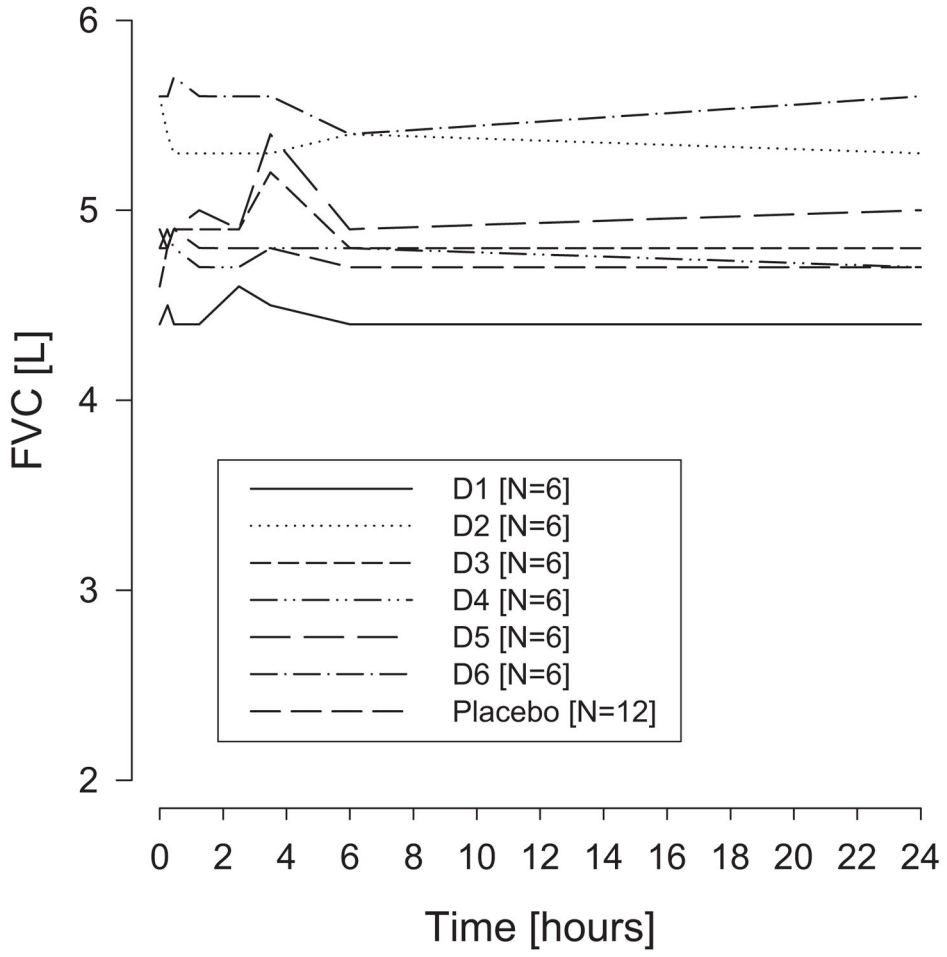

Figure 1.
Arithmetic mean baseline and post-dose FVC (L) values by AP301 dose group (N=6) and pooled placebo recipients (N=12).

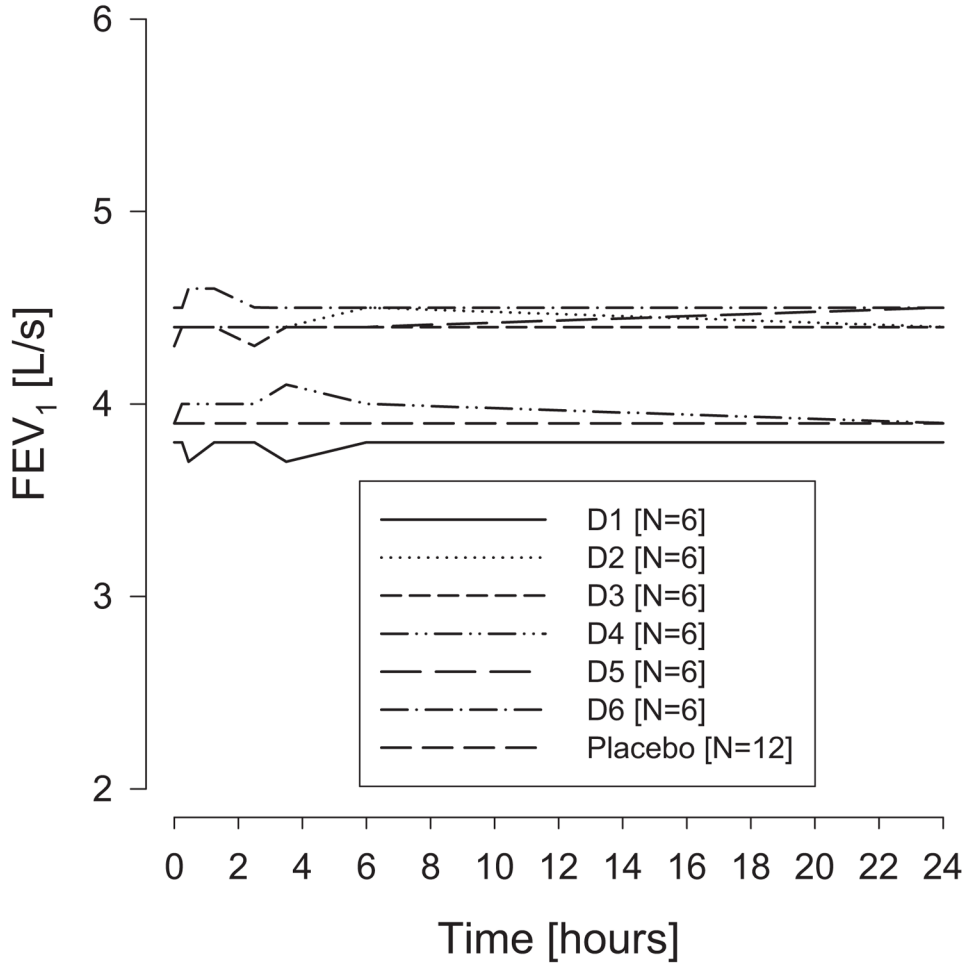

Figure 2.

Arithmetic mean baseline and post-dose FEV_1 (L) values by AP301 dose group (N=6) and pooled placebo recipients (N=12).

Table 1

Summary of Dosing Details, i.e., Nebulizer Filling Volumes (i.e., Metered AP301/Placebo Solution Volumes), AP301 Nebulizer AP301 Filling Dose (i.e., Metered Doses), Estimated Orally Delivered Doses (i.e., Ex Nebulizer Mouthpiece), and Estimated Respirable Dose Fraction (i.e., Dose Estimated to be Deposited in the Airways/Lungs)

Treatment/Dose	Group	Nebulizer Filling Volume [mL]	Nebulizer Filling Dose [mg]	Orally Delivered Dose [mg]	Estimated Dose Deposited in Lung [mg]	Dose Escalation Factor vs. Preceding Orally Delivered Dose
Group 1	D1	0.30	6.0	4.32	2.58	n.a.
Group 2	D2	0.70	17.4	12.0	7.20	2.8
Group 3	D3	1.70	42.8	30.0	18.0	2.5
Group 4	D4	3.40	85.7	60.0	36.0	2.0
Group 5	D5	5.20	128.5	90.0	54.0	0.5
Group 6	D6	6.90	171.4	120.0	72.0	0.3

Table 2

Subjects Demographic Characteristics at Baseline (Randomized Safety and PK Population, N = 48)

	Safety and PK Population (N = 48)						
	Dose Groups						
Demographic Data	**D1 4.32 mg, N = 6**	**D2 12 mg, N = 6**	**D3 30 mg, N = 6**	**D4 60 mg, N = 6**	**D5 90 mg, N = 6**	**D6 120 mg, N = 6**	***P*, N = 12**
Age (years)							
Mean ± SD	32.3 ± 9.2	30.3 ± 2.9	26.8 ± 4.0	29.0 ± 7.4	24.0 ± 1.5	27.3 ± 7.1	28.8 ± 7.6
Median (range)	33.0 (21–42)	30.5 (26–35)	28.0 (21–31)	26.5 (23–43)	24.5 (21–25)	25.5 (22–41)	27.0 (22–44)
Body weight (kg)							
Mean ± SD	71.1 ± 5.2	77.3 ± 5.4	70.7 ± 7.6	77.6 ± 6.1	68.9 ± 6.2	76.8 ± 3.9	71.3 ± 6.1
Median (range)	69.5 (65.8–79.0)	76.5 (72.0–85.0)	70.8 (62.0–81.0)	78.0 (71.0–84.8)	69.0 (60.0–77.5)	77.0 (69.9–81.0)	71.0 (61.0–84.9)
Height (cm)							
Mean ± SD	174.8 ± 4.2	179.0 ± 5.4	183.8 ± 7.3	178.0 ± 5.7	182.2 ± 6.5	182.3 ± 4.3	177.3 ± 6.1
Median (range)	176.0 (168–180)	178.5 (170–186)	186.5 (175–193)	180.5 (169–183)	182.5 (172–190)	183.0 (177–187)	176.0 (164–188)
BMI							
Mean ± SD	23.3 ± 1.7	24.1 ± 1.3	20.9 ± 1.2	24.5 ± 1.6	20.7 ± 0.5	23.2 ± 1.7	22.7 ± 1.4
Median (range)	23.3 (21.4–25.5)	24.3 (22.7–25.4)	20.6 (20.1–23.2)	24.9 (21.7–26.3)	20.5 (20.3–21.5)	23.8 (20.2–24.6)	22.6 (20.1–24.8)
Race							
Caucasian, n (%)	6 (100%)	5 (83.3%)	5 (83.3%)	5 (83.3%)	5 (83.3%)	6 (100%)	11 (91.7%)
Asian, n (%)	0	1 (16.7%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	0	1 (8.3%)

Table 3

Summary of Key AP301 PK Characteristics Calculated From Observed AP301 Plasma Concentrations After the Single Dose Inhalation of 4.32 (D1), 12 (D2), 30 (D3), 60 (D4), 90 (D5), and 120 mg (D6) of AP301 (Arithmetic Means ± SD for C_{max} and AUC_{0-t}; Medians (Ranges) for t_{max}; N=6/Group and Variable Unless Otherwise indicated)

Delivered Dose (mg)	Cmax (ng/mL)	AUC_{0-t} (ng × min/mL]	t_{max} (min)
4.32	LLOQ	LLOQ	NC
12	0.4 ± 0.6	2.0 ± 3.8[a]	5.0 (5.0–5.0)
30	0.3 ± 0.6	1.1 ± 2.7[b]	0 (0–0)
60	2.2 ± 1.4	55.8 ± 54.9[c]	5.0 (5.0–20.0)
90	2.4 ± 2.1	45.5 ± 39.9[d]	0 (0–5.0)
120	2.2 ± 1.8	52.6 ± 66.0	2.5 (0.0–20.0)

LLOQ, below the lower limit of quantification; NC, not calculated.

[a] N = 2.

[b] N = 1.

[c] N = 5.

[d] N = 4.