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ARTICLE

Dose-dependent inactivation of airway tryptase with a novel dissociating anti-tryptase antibody (MTPS9579A) in healthy participants: A randomized trial

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Abstract

Tryptase is the most abundant secretory granule protein in human lung mast cells and plays an important role in asthma pathogenesis. MTPS9579A is a novel monoclonal antibody that selectively inhibits tryptase activity by dissociating active tetramers into inactive monomers. The safety, tolerability, pharmacokinetics (PKs), and systemic and airway pharmacodynamics (PDs) of MTPS9579A were assessed in healthy participants. In this phase I single-center, randomized, observer-blinded, and placebo-controlled study, single and multiple ascending doses of MTPS9579A were administered subcutaneously (s.c.) or intravenously (i.v.) in healthy participants. In addition to monitoring safety and tolerability, the concentrations of MTPS9579A, total tryptase, and active tryptase were quantified. This study included 106 healthy participants (82 on active treatment). Overall, MTPS9579A was well-tolerated with no serious or severe adverse events. Serum MTPS9579A showed a dose-proportional increase in maximum serum concentration (C_{max}) values at high doses, and a nonlinear increase in area under the curve (AUC) values at low concentrations consistent with target-mediated clearance were observed. Rapid and dose-dependent reduction in nasosorption active tryptase was observed postdose, confirming activity and the PK/PD relationship of MTPS9579A in the airway. A novel biomarker assay was used to demonstrate for the first time that an investigative antibody therapeutic (MTPS9579A) can inhibit tryptase activity in the upper airway. A favorable safety and tolerability profile supports further assessment of MTPS9579A in asthma. Understanding the exposure-response relationships using the novel PD biomarker will help inform clinical development, such as dose selection or defining patient subgroups.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
MTPS9579A is a novel monoclonal antibody that selectively inhibits tryptase activity by dissociating active tetramers into inactive monomers. In preclinical studies, MTPS9579A inhibited tryptase activity in the airway.

WHAT QUESTION DID THIS STUDY ADDRESS?
This phase I study reports the safety and tolerability, pharmacokinetics (PKs), pharmacodynamics, and dose response of MTPS9579A in healthy human participants.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
MTPS9579A was well-tolerated at all tested dose levels and had linear PKs at high concentrations. MTPS9579A is pharmacologically active and inhibits the target (active tryptase) in the upper airway of healthy participants.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
These results allow further exploration into the clinical efficacy and optimization of doses for MTSP9579A in treating patients with asthma and other mast cell-related diseases.

INTRODUCTION

Tryptase, the most abundant secretory granule protein in human mast cells,[1,2] is a member of the serine protease family and is enzymatically active only in the tetrameric form. Tetrameric tryptase is assembled in mast cell granules in the presence of heparin and an acidic environment, and is released upon mast cell degranulation.[3–5] Mast cells and tryptase have been implicated in many pathophysiological conditions, including asthma and inflammation.[6] Catalytically active, extracellular secreted tryptase promotes airway hyper-responsiveness, bronchoconstriction, and amplification of mast cell degranulation.[7] Tryptase levels are elevated in bronchoalveolar lavage (BAL) fluid and serum of patients with asthma and correlate with disease severity.[8]

Multiple small molecule inhibitors block tryptase activity by targeting its active site, and some have shown efficacy in rodent and sheep models of allergic asthma.[9–11] In a human phase II allergen challenge study, the tryptase small molecule inhibitor APC 366 attenuated the allergen-induced late asthmatic response in patients with mild atopic asthma.[12] However, at certain dosages, some patients given nebulized APC 366 exhibited signs of bronchospasm at a higher frequency than those in the placebo group. Small molecule inhibitors of tryptase, including APC 366, have been terminated because of specificity, potency, or toxicity issues, so additional methods of blocking tryptase in clinical studies are needed to assess the role of tryptase in disease.[12]

MTPS9579A is a full-length humanized immunoglobulin (Ig) G4 antibody that binds with high affinity to human and cynomolgus monkey tryptase. MTPS9579A inhibits tryptase activity by irreversibly dissociating the active tetramer into inactive monomers. In a humanized mouse model, MTPS9579A treatment reduced IgE-mediated systemic anaphylaxis by blocking tryptase activity.[8] Treatment with MTPS9579A also significantly reduced active tryptase levels in the lungs of cynomolgus monkeys in an inhaled allergen challenge model.[8] MTPS9579A has also shown favorable nonclinical pharmacokinetics (PKs) and may be a therapeutic candidate for severe asthma and other mast cell-related disorders.

This first-in-human phase I study evaluated the safety, tolerability, PKs, and pharmacodynamics (PDs) of single and multiple ascending doses of MTPS9579A in healthy participants. Key objectives were to determine whether sufficient quantities of MTPS9579A distributed to the airway to effectively inhibit the target, tryptase activity, and to characterize the PK/PD relationship. Nasosorption, a noninvasive method that collects mucosal lining fluid from the nose (upper airway), was used to sample the upper airway, and a novel assay that specifically measures the active tetrameric form of tryptase was used to evaluate target inhibition in nasosorption.[13] Overall, MTPS9579A treatment-induced dose-dependent decreases in active tryptase levels in the upper airway of healthy participants and was safe and well-tolerated with favorable PKs at high doses.

METHODS

Trial design

This phase I, randomized, observer-blinded, placebo-controlled, single and multiple ascending dose (SAD, MAD)

study was conducted in two parts (Figure S1) at a single clinical site in Canada (inVentiv Health Clinical). Part A consisted of seven SAD cohorts of healthy participants who received the following subcutaneous (s.c.) or intravenous (i.v.) doses of MTPS9579A or matching placebo: cohort A = 30 mg s.c., cohort B = 100 mg s.c., cohort C = 300 mg s.c., cohort D = 300 mg i.v., cohort E = 900 mg i.v., cohort I = 1800 mg i.v., and cohort J = 3600 mg i.v. Part B consisted of five MAD cohorts of healthy participants who each received a total of three doses of MTPS9579A or matching placebo once every 4 weeks (q4w): as cohort F = 150 mg s.c., cohort G = 300 mg s.c., cohort H = 750 mg s.c., cohort L = 1350 mg i.v., and cohort M = 3600 mg i.v. The treatment allocation for these cohorts was six active: two placebo for the SAD and eight active: two placebo for the MAD.

The initial dose for MTPS9579A was selected based on the no observed adverse effect level (NOAEL) of 100 mg/kg administered either i.v. or s.c. in a 13-week, repeat-dose cynomolgus monkey toxicity study (data not shown). The highest dose tested during the toxicity study was 100 mg/kg. Based on the NOAEL of 100 mg/kg i.v., the safety factors are greater than 98-fold for the starting dose of 30 mg s.c. and greater than 1.2-fold for the highest dose of 3600 mg i.v. based on area under the curve (AUC). A safety monitoring committee reviewed all available clinical and safety data prior to dose escalation to subsequent cohorts.

Ethics

The clinical study protocol, any relevant associated documents, and informed consent forms (ICFs) were reviewed and approved by an institutional review board (IRB; IRB Services; Advarra) before beginning study procedures. All clinical work was conducted in compliance with Good Clinical Practices (GCP) and Good Laboratory Practices according to the International Conference on Harmonization (ICH) guidelines (ICH E6), and all applicable regulations, including the Federal Food, Drug, and Cosmetic Act, US applicable Code of Federal Regulations (CFR) Title 21, and any independent ethics committee (IEC) requirements relative to clinical studies. The study was also conducted in compliance with the recommendations in the Declaration of Helsinki, with the exception that registration of phase I trials in a publicly accessible database was not mandatory. All participants provided written informed consent.

Participants

Eligible participants were healthy adult men and non-pregnant women, 18–55 years old, with a body mass index (BMI) of 18–32 kg/m^2, a body weight greater than or equal to 60.0 kg, and in good general health, as determined by medical history, physical examination, and routine clinical laboratory tests. Participants with a recent smoking history, significant allergic disease including asthma, or positive tuberculin skin test were excluded.

Treatment assignment and blinding

Participants were randomly assigned numbers that corresponded to the previously generated randomization scheme generated by inVentiv using validated computer software (SAS, version 9.2; SAS Institute) and was reviewed by a biostatistician.

The study was observer-blinded. All participants and study site personnel (with the exception of the unblinded pharmacist) were blinded to treatment assignment throughout the study unless unblinding was required to resolve an adverse event (AE).

Safety assessments

The safety and tolerability of single and multiple doses of MTPS9579A in healthy participants was assessed by determining the frequency and severity of AEs, with severity determined according to the Division of Microbiology and Infectious Diseases (DMID) toxicity scale,[14] and the change from baseline in targeted vital signs, clinical laboratory test results, and 12-lead electrocardiogram (ECG) parameters. The safety population comprised all participants who received at least one dose of study drug (MTPS9579A or placebo). AEs were reported starting with initiation of study drug and continuing until 84 days after the last dose. Vital signs included measurements of respiratory rate, pulse rate, pulse oximetry, systolic and diastolic blood pressure while the subject was in a seated position (resting for at least 5 min), and oral temperature. Standard hematology, chemistry, and coagulation parameters were measured at the study site's local laboratory.

Immunogenicity

The numbers and proportions of anti-drug antibody (ADA)-positive participants and ADA-negative participants at baseline (baseline prevalence) and after drug administration (postbaseline incidence) were summarized by treatment groups. A tiered strategy was used to detect and characterize anti-MTPS9579A antibodies. Serum samples were first screened in a bridging screening assay. Samples that screened positive were further analyzed by

competitive binding with MTPS9579A to confirm the positive response in the assay. Confirmed-positive samples were diluted further to obtain a value in titer units.

When determining postbaseline incidence, participants were considered to be ADA positive if they were ADA negative or had missing data at baseline but developed an ADA response following study drug exposure (treatment-induced ADA response), or if they were ADA positive at baseline and the titer of one or more postbaseline samples was at least 0.60 titer units greater than the titer of the baseline sample (treatment-enhanced ADA response). Participants were considered to be ADA negative if they were ADA negative or had missing data at baseline and all postbaseline samples were negative, or if they were ADA positive at baseline but had no postbaseline samples with a titer that was at least 0.60 titer units greater than the titer of the baseline sample (treatment unaffected). Persistence and transience were defined according to Shankar et al.[15] SAD samples were collected predose day 1, day 29, day 57, and day 85 of study conduct. MAD samples were collected predose day 1, day 29, day 43, day 57, day 113, and day 141 of study conduct.

Pharmacokinetic outcome measures

To characterize the PK profile of MTPS9579A following s.c. or i.v. dosing, serum samples for part A were collected on days 1, 2, 5, 8, 15, 29, 43, 57, and 85 or at early termination and for part B, on days 1, 2, 5, 8, 15, 29, 43, 57, 61, 71, 85, 113, and 141 or at early termination. These timepoints were chosen based on predicted PK parameters of MTPS9579A from nonclinical studies and knowledge of IgG monoclonal antibodies. A direct sandwich enzyme-linked immunosorbent assay (ELISA) measured MTPS9579A concentrations in serum. Target interference within the PK assay was evaluated for up to 4000 ng/ml of tryptase at the lower limit of quantification (LLOQ; 250 ng/ml MTPS9579A) of the PK assay. No interference to the assay was observed at these levels (molar ratio of target to drug ~100:1). Inter-run precision and accuracy, acceptance criteria were set at ±20%, total error below 30% and are summarized in Table S1.

PK assessments of available data for all cohorts were performed for serum concentration-time data using standard noncompartmental analysis (NCA) methods and Phoenix WinNonlin software, version 8.0 (Pharsight Corporation). The following PK parameters were evaluated: maximum serum concentration (C_{max}), lowest serum concentration prior to next dose (C_{trough}), clearance (CL), volume of distribution (V), AUC, area under the concentration-time curve during the dosing interval of 0–28 days (AUC_{tau}), terminal half-life ($t_{1/2}$), and time to maximum serum concentration (T_{max}). Exploratory PK outcomes included assessing the relationship between drug exposure and PD biomarkers.

Nasosorption sampling

Nasosorption is a noninvasive, upper airway sampling method that uses a synthetic absorptive matrix (SAM) to collect mucosal lining fluid from the nasal cavity. Nasosorption samples were collected from participants using the Nasosorption Fx-i device (Hunt Developments). The device was inserted into the nostril, not passing the inner nares, and held against the side of the nostril for 60 s to collect mucosal lining fluid onto the SAM. Mucosal lining fluid was subsequently eluted from the SAM by immersing the SAM in 300 μl of elution buffer (50 mM Tris-HCL, pH 7.4, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 0.05% Tween 20, 1 mM sodium orthovanadate, 1% bovine serum albumin (BSA), and 0.05% ProClin 300 in a spin filter (E&K Scientific) for 30 min on ice. Samples were centrifuged at 16,000 × *g* for 15 min at 4°C and supernatants were collected for sample analysis.[16]

Pharmacodynamic outcome measures

Exploratory outcomes included measurement of active and total tryptase, PD biomarkers that could provide evidence of MTPS9579A target engagement. Serum samples for part A were collected on days 1 (predose), 2, 5, 8, 15, 29, 43, 57, and 85 and for part B on days 1 (predose), 5, 15, 71, 85, 113, and 141. Nasosorption samples for part A were collected on days 1 (predose), 2, 5, 15, and 29, and for part B on days 1 (predose), 57 (predose), 71, and 85. These timepoints were chosen to have paired biomarker data with key PK timepoints. Total tryptase was measured in serum and nasosorption samples by ELISA (LLOQ: 975 pg/ml) and Gyrolab Technology assay (LLOQ: 366 pg/ml; Gyros US), respectively; monoclonal antibodies used for capture and detection were generated at Genentech. An activity-based probe immunoassay on the Simoa platform specifically measured active tryptase in nasosorption (LLOQ: 40 pg/ml).[13] Samples below the LLOQ were imputed as LLOQ/2 for each assay. Nasosorption data were missing for some participants and/or timepoints because of insufficient sample volume available; no imputation was used for samples with missing data.

Statistical methods

Statistical summaries were descriptive (e.g., incidence rates, means, and percentiles). Participants were grouped

according to treatment received, and who received any amount of MTPS9579A or placebo were included in the analyses. All participants assigned to placebo (regardless of dose group) were combined into a single placebo control group corresponding to part A or part B. Descriptive statistics (mean, median, SD, minimum [min], and maximum [max]) were calculated for continuous variables. Frequency counts and percentages were tabulated for categorical variables. The Medical Dictionary for Regulatory Activities (MedDRA), version 21.0, was used to classify all AEs by System Organ Class (SOC) and Preferred Term (PT). Laboratory data were listed, with values outside of normal ranges identified.

PD biomarkers (active and total tryptase) were assessed to determine the pharmacological activity and mechanism of action of MTPS9579A. Data were summarized by absolute biomarker levels, and absolute and relative changes from baseline (defined as predose) for each treatment group. Biomarker values below the LLOQ were imputed as LLOQ/2.

Serum PK analyses included participants with sufficient data to enable estimation of key parameters (e.g., AUC, C_{max}, and $t_{1/2}$). Mean serum MTPS9579A concentration-versus-time data was tabulated and plotted by dose level. Estimates for parameters were tabulated and summarized (geometric mean, SD, coefficient of variation [CV], median, minimum, and maximum). Intersubject variability and drug accumulation were evaluated.

RESULTS

Study overview

In total, we enrolled and randomized 106 healthy adults. The first subject received their first dose on March 19, 2018 and the last patient's last visit was on April 12, 2019. Of the 106 enrolled, all participants received at least one dose of study drug (MTPS9579A or placebo), and 104 participants completed the study, including the follow-up visits. Forty-two participants and 40 participants in the SAD and MAD, respectively, received MTPS9579A (Table 1). Age, sex, race, ethnicity, and baseline weight were generally well balanced between MTPS9579A-treated participants and placebo-treated participants in both portions of the study.

Safety

MTPS9579A was well-tolerated in healthy participants when administered as either a single s.c. or i.v. dose over the range of 30 to 3600 mg and following multiple s.c. or i.v. doses q4w from 150 to 3600 mg. In the combined SAD and MAD, 82 (77.4%) of the 106 participants in the safety population reported a total of 413 treatment-emergent AEs (TEAEs; Table 2; Tables S2 and S3): 63 (76.8%) of 82 participants who received MTPS9579A reported 339 TEAEs and 19 (79.2%) of 24 participants who received placebo reported 74 TEAEs. In both the SAD and MAD portions of the study, the frequencies and severities of TEAEs were similar between cohorts and between participants randomized to MTPS9579A and those randomized to placebo. There were no deaths, or serious or life-threatening AEs during the study. The majority of observed TEAEs were grade 1 (mild) in severity and considered not related to the study drug. Only one subject discontinued because of a TEAE (blood creatine phosphokinase increased) during part B (MAD). The most common TEAEs were nasopharyngitis, headache, and injection site erythema; these were generally balanced between treatment groups (Table 2, Tables S2 and S3).

There was no pattern in the development or magnitude of laboratory abnormalities between cohorts or between participants who received MTPS9579A and placebo. No clinically significant abnormalities were observed with respect to ECG results. No relevant differences were observed between the treatment groups for clinical laboratory results, vital signs, and ECG results.

Pharmacokinetics

For cohorts A, B, and C (30, 100 and 300 mg, respectively), after s.c. administration, we observed the same T_{max} regardless of dose level (median T_{max} ~7 days; Figure 1a, Table S4). Clearance after MTPS9579A treatment ranged from 0.357 to 0.568 L/day after single-dose s.c. administration and from 0.133 to 0.189 L/day after single-dose i.v. administration (Table S4). Mean half-life values were generally shorter at the lower doses (30–300 mg s.c.). At high doses (1800–3600 mg i.v.), the $t_{1/2}$ of the linear range was calculated to be 30–35 days by NCA. Decreasing clearances and longer half-life values with increases in dose suggested nonlinear PK, possibly due to target-mediated clearance at lower anti-tryptase serum concentrations.

Similar PK findings were observed after multiple ascending doses were studied in part B (Figure 1b, Table S5). After the third and final dose administration in the 1350 and 3600 mg i.v. cohorts, MTPS9579A exposure increased approximately dose proportionally, with a 3.29-fold increase in C_{max} (Table S5) compared to a 2.7-fold increase in dose. The mean accumulation ratio (AR) after multiple s.c. q4w doses ranged from 1.2 to 2.0 and was similar after multiple i.v. q4w dose cohorts (AR = 1.9–2.3), when comparing the AUC_{tau}. After multiple i.v. doses

TABLE 1 Participant demographics and baseline characteristics in the SAD and MAD stages

SAD	All placebo ($n = 14$)	All MTPS9579A participants ($n = 42$)	A 30 mg s.c. ($n = 6$)	B 100 mg s.c. ($n = 6$)	C 300 mg s.c. ($n = 6$)	D 300 mg i.v. ($n = 6$)	E 900 mg i.v. ($n = 6$)	I 1800 mg i.v. ($n = 6$)	J 3600 mg i.v. ($n = 6$)
Age (y), mean (SD)	37.6 (12.1)	38.1 (9.3)	41.5 (10.4)	36.5 (3.9)	40.3 (7.5)	40.0 (10.8)	36.2 (11.8)	31.8 (10.0)	40.2 (9.2)
Sex, female, *n* (%)	10 (71.4%)	26 (61.9%)	3 (50.0%)	5 (83.3%)	4 (66.7%)	6 (100%)	3 (50.0%)	4 (66.7%)	1 (16.7%)
Race, *n* (%)									
Asian	1 (7.1%)	1 (2.4%)	0	0	1 (16.7%)	0	0	0	0
Black or African American	0	1 (2.4%)	0	0	0	1 (16.7%)	0	0	0
White	13 (92.9%)	40 (95.2%)	6 (100%)	6 (100%)	5 (83.3%)	5 (83.3%)	6 (100%)	6 (100%)	6 (100%)
Ethnicity, *n* (%)									
Hispanic or Latino	4 (28.6%)	5 (11.9%)	2 (33.3%)	0	0	1 (16.7%)	0	2 (33.3%)	0
Weight (kg), mean (SD)	75.57 (12.66)	73.89 (14.62)	70.87 (14.88)	73.32 (14.31)	71.90 (16.07)	63.67 (12.81)	82.32 (17.21)	72.82 (8.15)	82.33 (15.08)

MAD	All Placebo ($n = 10$)	All MTPS9579A participants ($n = 40$)	F 150 mg s.c. ($n = 8$)	G 300 mg s.c. ($n = 8$)	H 750 mg s.c. ($n = 8$)	L 1350 mg i.v. ($n = 8$)	M 3600 mg i.v. ($n = 8$)
Age (y), Mean (SD)	36.0 (8.5)	39.4 (8.8)	34.0 (7.6)	42.3 (7.7)	44.8 (10.6)	37.1 (8.9)	38.9 (6.5)
Sex, female, *n* (%)	8 (80.0%)	26 (65.0%)	4 (50.0%)	7 (87.5%)	6 (75.0%)	3 (37.5%)	6 (75.0%)
Race, *n* (%)							
White	10 (100%)	40 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
Ethnicity, *n* (%)							
Hispanic or Latino	1 (10.0%)	5 (12.5%)	1 (12.5%)	1 (12.5%)	0	3 (37.5%)	0
Weight (kg), mean (SD)	66.91 (6.72)	72.36 (12.34)	66.78 (10.99)	68.86 (10.79)	73.63 (15.02)	81.71 (13.25)	70.80 (7.47)

Abbreviations: MAD, multiple ascending dose; SAD, single ascending dose.

TABLE 2 Overview of safety

	SAD		MAD	
	All placebo ($n = 14$)	**All MTPS9579A ($n = 42$)**	**All placebo ($n = 10$)**	**All MTPS9579A ($n = 40$)**
Total number of participants with at least one AE	11 (78.6%)	27 (64.3%)	8 (80.0%)	36 (90.0%)
Total number of AEs	25	71	49	268
Total number of deaths	0	0	0	0
Total number of participants withdrawn from study due to an AE	0	0	0	0
Total number of participants with at least one				
SAE	0	0	0	0
AE leading to withdrawal from treatment	0	0	0	1 (2.5%)
Related AE	9 (64.3%)	22 (52.4%)	8 (80.0%)	33 (82.5%)

Abbreviations: AE, adverse event; MAD, multiple ascending dose; SAD, single ascending dose; SAE, serious AE.

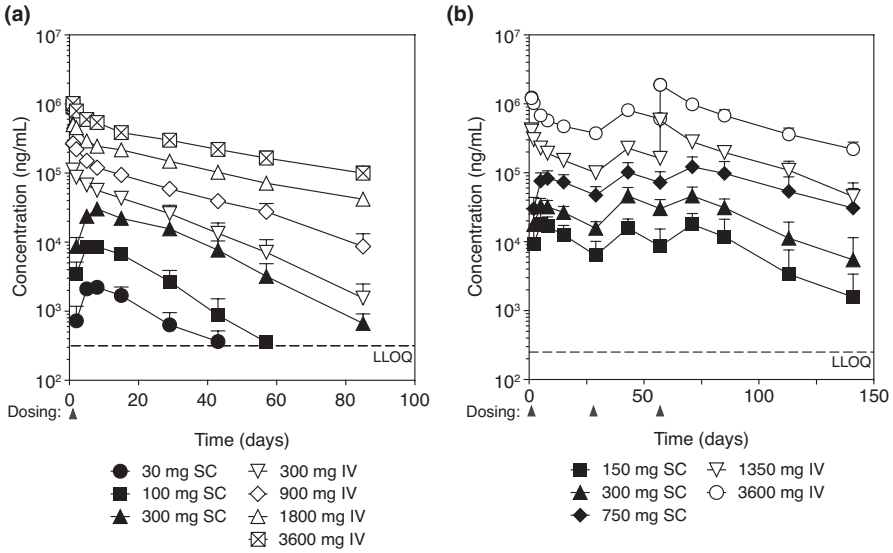

FIGURE 1 Observed serum concentrations after single or multiple doses of MTPS9579A. Mean (+SD) serum MTPS9579A concentrations were analyzed over time. (a) A single ascending dose was administered on day 1 and pharmacokinetic concentrations were analyzed for 84 days post-dose. (b) Multiple ascending doses were administered on days 1, 29, and 57, and serum MTPS9579A concentrations were analyzed through day 141. Samples below LLOQ (250 ng/ml) were not included. LLOQ, lower limit of quantification

(1350 or 3600 mg), $t_{1/2}$ was calculated as 25 and 34 days, respectively.

Immunogenicity

Overall, none of the 106 participants in the SAD or MAD were positive for ADAs at baseline (Table S6). Among those who received MTPS9579A, 4 of 42 (9.5%) participants in the SAD cohort developed ADAs, and 2 of 40 (5%) participants in the MAD cohort developed ADAs. One participant who received placebo in the SAD had a post-baseline, ADA-positive sample at a single timepoint, with a low, transient titer. The ADA positivity of this sample was attributed to the false-positive rate designed into the ADA assay. ADA incidence in MTPS9579A-treated participants was low and did not affect serum exposures of MTPS9579A significantly.

Pharmacodynamics (total tryptase)

After MTPS9579A binds to and dissociates the active tetramer, it remains bound to the inactive monomeric form of tryptase. Antibody binding to the monomeric form is predicted to increase its half-life, resulting in elevations of tryptase observed. To determine if MTPS9579A binds to tryptase in vivo, we measured serum and nasosorption

levels of total tryptase. Baseline levels of serum total tryptase ranged from 2.3 to 19.7 ng/ml (mean of 7.7 ng/ml), similar to levels reported in literature.[8,17] Absolute levels of total tryptase in serum increased over time in participants who received MTPS9579A but not in those who received placebo (Figure 2). Within each cohort, peak postdose total tryptase levels were variable across individuals; however, there was a dose-dependent elevation observed across cohorts. Serum tryptase levels peaked around 14 days postdose. In higher dose cohorts (i.e., SAD cohort D and MAD cohort G), postdose tryptase levels plateaued and remained elevated throughout the sampling period.

Compared with serum, nasosorption samples exhibited total tryptase levels that were more variable at baseline. We therefore present the percent change from baseline to better visualize any postdose increases (Figure 3). Despite the variability in postdose increases in total tryptase in the nasosorption, there was a trend toward increases in total tryptase in participants who received MTPS9579A but not in those who received placebo. Tryptase kinetics may be different in tissue than serum, which may explain why the effect of antibody stabilization and the magnitude of postdose tryptase elevations were different between serum and nasosorption samples.

Pharmacodynamics (active tryptase)

We measured levels of active tryptase in nasosorption samples to investigate the effects of MTPS9579A on tryptase activity in healthy participants. Active tryptase was not measured in serum because the active tetramer is unstable and thought to fall apart before reaching circulation. In samples collected prior to dosing (baseline), active tryptase was detectable in the majority (72%) of participants (Figure 4). In placebo-treated participants, active tryptase levels remained relatively stable over the course of the study. However, levels of active tryptase fell below the LLOQ after MTPS9579A administration in cohorts B, C, D, E, I, and J in the SAD group (Figure 4a) and cohorts G, H, L, and M in the MAD group (Figure 4b). In the SAD group, we also observed a rapid decline of active tryptase in nasosorption samples collected at early time points following dosing (day 2 for i.v. and day 5 for s.c. were the earliest postdose timepoints collected for these routes of administration). In SAD cohorts D, E, I, and J, these PD effects were sustained through day 29; in the MAD cohorts G, H, L, and M, these PD effects were sustained between doses and for 28 days after the last dose. These data demonstrate that MTPS9579A is pharmacologically active and inhibits the target (active tryptase) in the upper airway of healthy participants.

PK/PD analysis of active tryptase

We performed a PK/PD analysis to determine the relationship between active tryptase levels and MTPS9579A exposures. Serum PK samples were matched to the respective nasosorption samples collected at 28 days after the final dose (corresponding to C_{trough}; SAD group day 28 and MAD group day 84). An exposure-response relationship was observed in both the SAD (Figure 5a) and the MAD (Figure 5b) studies. The MAD PKs was generally higher than the SAD, as expected, with few low values. Both portions of the study (SAD and MAD) resulted in inhibition of the nasosorption active tryptase at high serum PK concentrations. Due to low subject numbers per cohort and variable data in SAD and MAD, additional studies will be needed to further define the extent of inhibition at lower serum concentrations and refine this exposure-response relationship; however, this analysis further indicates MTPS9579A inhibits active tryptase.

DISCUSSION

MTPS9579A is an investigational anti-tryptase antibody therapeutic in development for asthma. In healthy participants, MTPS9579A was well-tolerated at all tested dose levels and had favorable PKs, especially at higher dose levels. Because of the novel mechanism of action of the antibody (inactivation of tryptase by disruption of the active tetrameric form into an inactive monomeric form), it was important to demonstrate pharmacological activity early in the clinic. MTPS9579A treatment was associated with a rapid and dose-dependent reduction in active tryptase levels and a corresponding dose-dependent increase in serum total tryptase levels, suggesting that MTPS9579A binds and inactivates tryptase.

There were no significant concerns about the safety profile of MTPS9579A in healthy participants. The SAD and MAD cohorts had similar frequencies and severities of TEAEs between cohorts and between participants on MTPS9579A and those on placebo. Development of earlier tryptase inhibitors have been terminated due to specificity, toxicity, and/or potency issues, even though these molecules have demonstrated proof of activity and concept.[12] Combining the favorable safety profile of MTPS9579A and early proof of target engagement enables further exploration of the hypothesis that tryptase is a key mediator in asthma and other mast cell-related diseases.

MTPS9579A displayed decreasing clearance and longer half-lives as dose levels increased, suggesting nonlinear PK at low s.c. doses (i.e., 30–300 mg). Although ADA may interfere in the PK assay, the ADA incidence in this phase I study was low. Furthermore, participants with a high-titer ADA response did not have lower MTPS9579A concentrations.

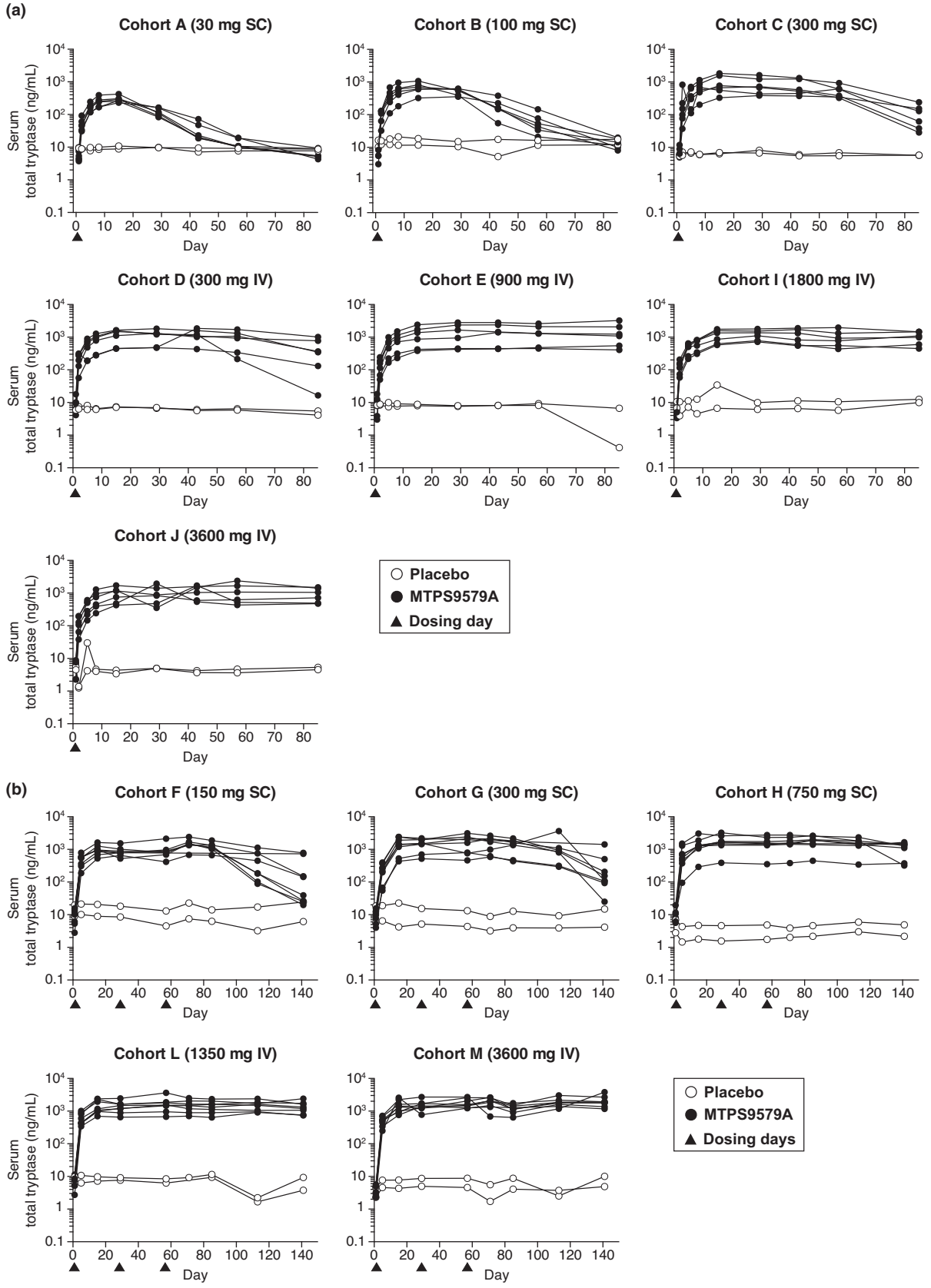

FIGURE 2 Dose-dependent increases in serum total tryptase after single or multiple doses of MTPS9579A. Participants received a single dose on day 1 (a) or multiple doses on days 1, 29, and 57 (b). On dosing days, serum was collected pre-dose

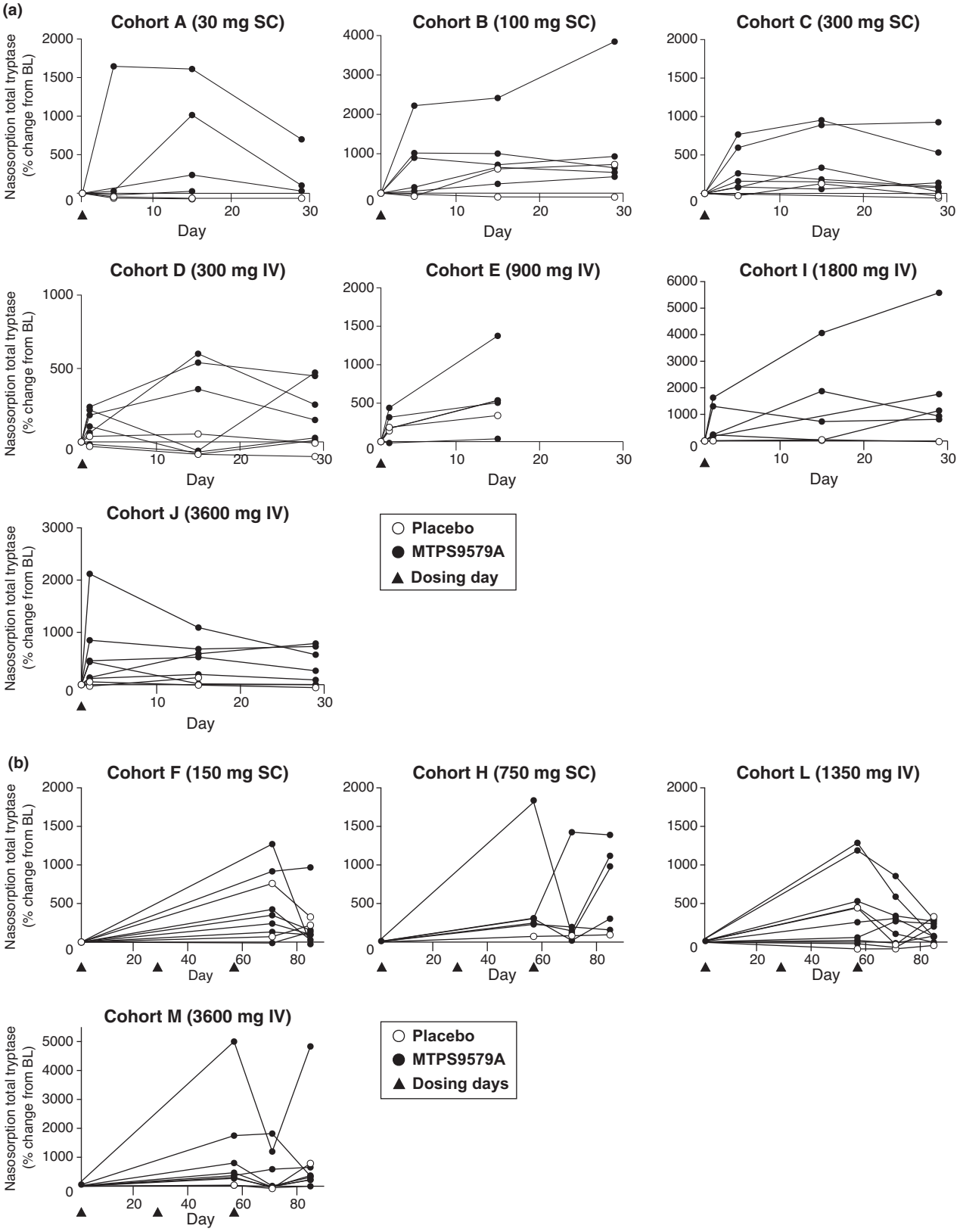

FIGURE 3 Increase in nasosorption total tryptase after single or multiple doses of MTPS9579A demonstrates target engagement in the airway. Participants received a single dose on day 1 (a) or multiple doses on days 1, 29, and 57 (b). On dosing days, nasosorption was collected predose. Data is missing for some timepoints because of insufficient sample volumes available

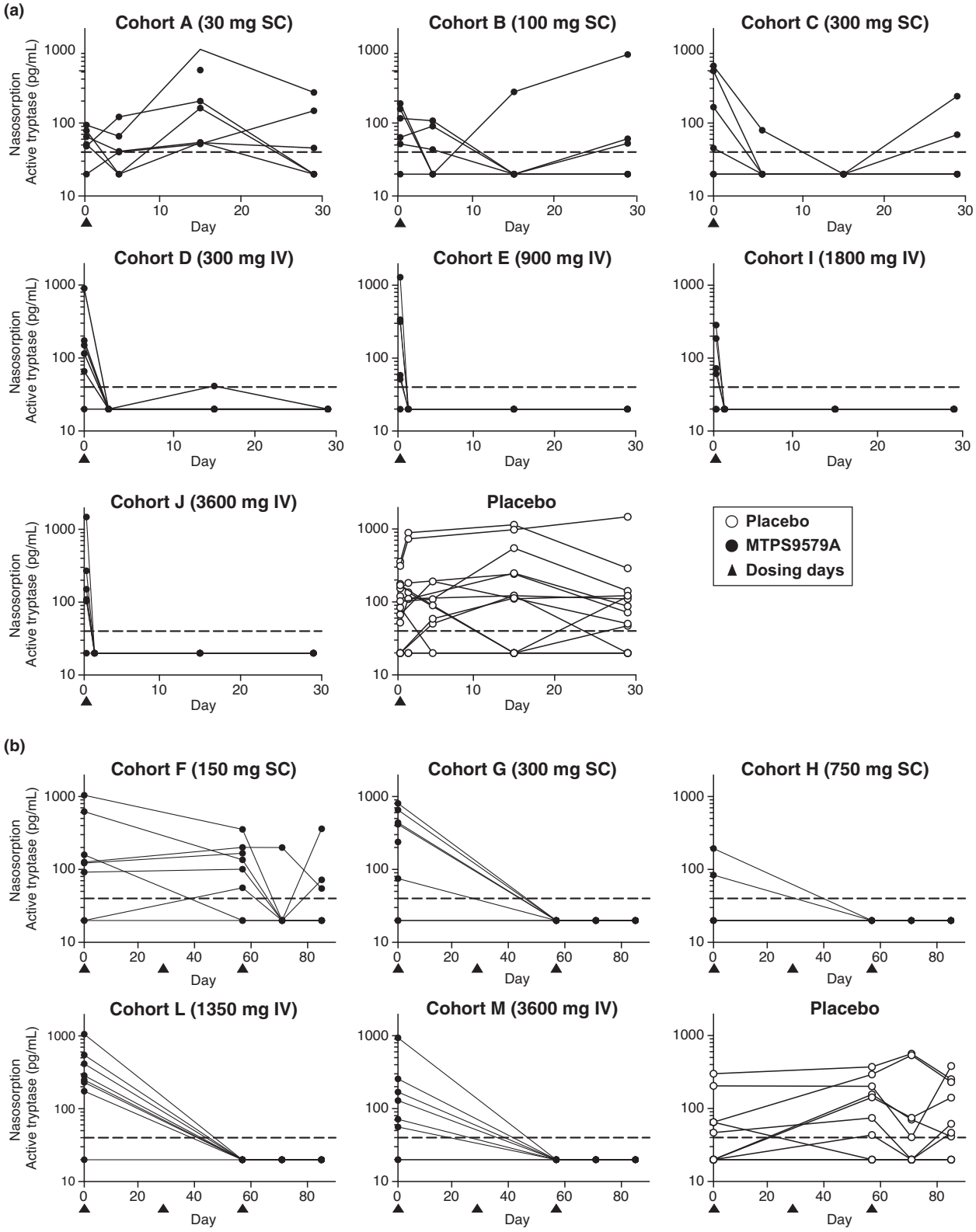

FIGURE 4 Dose-dependent reduction of nasosorption active tryptase after single or multiple doses of MTPS9579A demonstrates activity of MTPS9579A in the airway. Active tryptase levels were evaluated throughout study duration (a, b). Participants received a single dose on day 1 (a) or multiple doses on days 1, 29, and 57 (b). On dosing days, nasosorption was collected predose. Samples below LLOQ were imputed as LLOQ/2. BL, baseline; LLOQ, lower limit of quantification. Dashed line indicates LLOQ

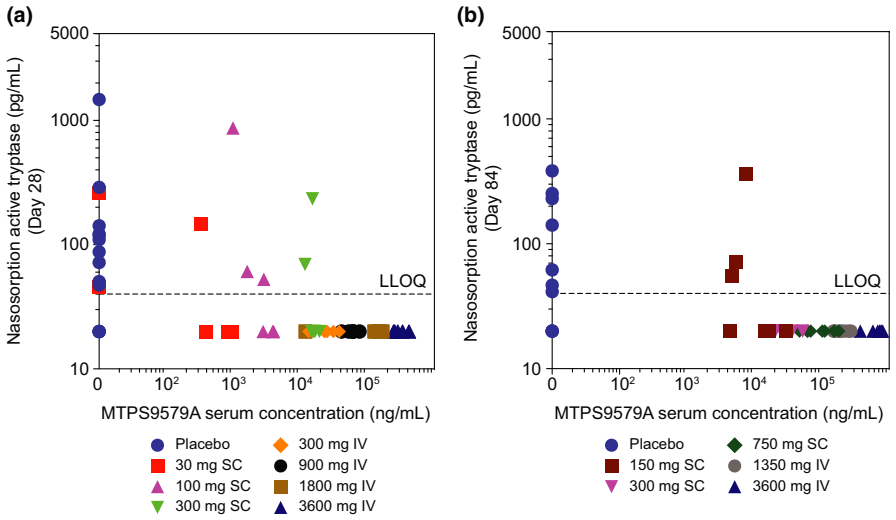

FIGURE 5 High MTPS9579A exposures correspond with no detectable nasosorption active tryptase. The relationship between serum MTPS9579A concentrations and nasosorption active tryptase at (a) 28 days post-single dose (SAD) or (b) 28 days post-third dose (MAD) were analyzed. Samples below LLOQ for active tryptase were imputed as LLOQ/2. SAD, single-ascending dose; LLOQ, lower limit of quantification; MAD, multiple-ascending dose; PK, pharmacokinetics

Within the PK assay, we observed no target interference at the highest levels of target tested, a molar ratio of ~100:1 (target:drug). Therefore, one plausible explanation for the observed nonlinear PK is target-mediated drug disposition (TMDD).[18] TMDD occurs when the binding of a high-affinity drug to its pharmacological target affects the drug's PK characteristics.[19] The combination of short half-life at low doses coupled with a dose-dependent increase in half-life is highly suggestive of TMDD. The dose-dependent increase of serum tryptase also suggests that circulating total serum tryptase saturates MTPS9579A at low doses (i.e., 30–300 mg s.c.), resulting in limited MTSP9579A available to bind to active tryptase, the intended target. At higher doses (i.e., 900–3600 mg i.v.), serum tryptase reaches a plateau, indicating complete saturation of serum tryptase and excess MTPS9579A in circulation. It also can explain the observed dose-proportional increases of MTPS9579A exposures at these higher doses and the expected long half-life of a monoclonal antibody. Knowing that total tryptase levels in patients with asthma are higher than healthy subjects, the concentration of MTPS9579A needed to saturate the total tryptase level before seeing decrease in active tryptase levels in asthmatic patients may be elevated. Further PK characterization using PK/PD modeling is warranted to confirm this hypothesis and aid dose selection for future studies.

The extent of target inhibition can depend on baseline tryptase levels, which can be highly variable between individuals, and vary with disease status and severity.[20] Tryptase levels are likely to be higher in disease populations than in healthy participants. For asthma and other airway diseases, tryptase levels may also differ between the upper and lower airways. In addition, mast cell activation and degranulation can occur during acute exacerbations of disease, potentially contributing to even higher levels of tryptase that must be inhibited in the target organ.[21] Because the lungs are the target organs and intended site of action of MTPS9579A, local MTPS9579A concentrations will be lower compared to serum; therefore, doses required to achieve adequate target inhibition may be higher in patients relative to healthy participants. Together, this highlights the need for PK/PD modeling and lower airway sampling.

Because levels of tryptase are key drivers of the PK/PD relationship and MTPS9579A has a unique pharmacology, further exploration in patient studies is crucial to understand whether the tryptase suppression observed in healthy participants can be extended to patients with asthma. A phase Ic clinical study (EudraCT: 2018-003562-14) has been initiated to measure active and total tryptase in both upper and lower airways to characterize the MTPS9579A pharmacology in patients with asthma. This study includes bronchoscopy sampling to focus on the intended site of action in patients, which is the lower airway. These data will support the interpretation of an ongoing phase IIa study (ClinicalTrials.gov: NCT04092582) to evaluate efficacy and safety in patients with asthma, and guide route of administration and dose selection for future asthma studies.

In summary, the phase I data demonstrate that MTPS9579A engages its target and substantially reduces active tryptase, while having an acceptable safety and tolerability profile. Multiple-dose PK characteristics were consistent with an IgG monoclonal antibody; exposures generally increased dose-proportionally at dose levels greater than 900 mg i.v. The low incidence of ADAs post-administration supports repeat dosing in a chronic disease population. Further characterization of MTPS9579A is ongoing in patients with asthma and will contribute to our understanding of the molecule.

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CONFLICT OF INTERESTS

S.M.R., S.S., G.S., M.B., K.Y., P.B., V. Sverkos, F.C., V. Steffen, L.M.H., J.G., H.R., P.N.B., J.H.L., and T.L.S are or were employees of Genentech, Inc., a member of the Roche Group, at the time this research was conducted and are Roche stockholders. J.S. was an employee of Roche at the time this research was conducted and is a Roche stockholder. S.S. is currently an employee of The Janssen Pharmaceutical Companies of Johnson & Johnson and is a stockholder of Johnson & Johnson. F.C. is currently an employee of AbbVie and is an AbbVie stockholder.

AUTHOR CONTRIBUTIONS

S.M.R., S.S., G.S., M.B., J.G., K.Y., J.S., P.B., V. Sverkos, F.C., V. Steffen, H.R., P.N.B., J.H.L., and T.L.S. wrote the manuscript. S.M.R., S.S., G.S., M.B., J.G., K.Y., J.S., F.C., V. Steffen, H.R., P.N.B., J.H.L., and T.L.S. designed the research. S.M.R., S.S., G.S., M.B., J.G., K.Y., J.S., P.B., V. Sverkos, F.C., V. Steffen, H.R., P.N.B., J.H.L., and T.L.S. performed the research. S.M.R., S.S., G.S., J.G., K.Y., J.S., F.C., V. Steffen, L.M.H., and T.L.S. analyzed the data. G.S. and M.B. contributed new reagents/analytical tools.

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SUPPORTING INFORMATION

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