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In vitro and clinical characterization of the valved holding chamber AeroChamber Plus® Flow-Vu® for administrating tiotropium Respimat® in 1–5-year-old children with persistent asthmatic symptoms

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ABSTRACT

Background: When characterizing inhalation products, a comprehensive assessment including *in vitro*, pharmacokinetic (PK), and clinical data is required. We conducted a characterization of tiotropium Respimat® when administered with AeroChamber Plus® Flow-Vu® anti-static valved holding chamber (test VHC) with face mask in 1–5-year-olds with persistent asthmatic symptoms.

Methods: *In vitro* tiotropium dose and particle size distribution delivered into a cascade impactor were evaluated under fixed paediatric and adult flow rates between actuation and samplings. The tiotropium mass likely to reach children's lungs was assessed by tidal breathing simulations and an ADAM-III Child Model. PK exposure to tiotropium in preschool children with persistent asthmatic symptoms (using test VHC) was compared with pooled data from nine Phase 2/3 trials in older children, adolescents, and adults with symptomatic persistent asthma not using test VHC.

Results: At fixed inspiratory flow rates, emitted mass and fine particle dose decreased under lower flow conditions; dose reduction was observed when Respimat® was administered by test VHC at paediatric flow rates. In < 5-year-old children, such a dose reduction is appropriate. In terms of dose per kg/body weight, *in vitro*-delivered dosing in children was comparable with adults. Transmission and aerosol holding properties of Respimat® when administered with test VHC were fully sufficient for aerosol delivery to patients. At zero delay, particles < 5 µm (most relevant fraction) exhibited a transfer efficacy of ≥60%. The half-time was > 10 s, allowing multiple breaths. Standardized tidal inhalation resulted in an emitted mass from the test VHC of approximately one-third of labelled dose, independent of coordination and face mask use, indicating predictable tiotropium administration by test VHC with Respimat®. Tiotropium exposure in 1–5-year-old patients using the test VHC, when adjusted by height or body surface, was comparable with that in older age groups without VHCs; no overexposure was observed. Adverse events were less frequent with tiotropium (2.5 µg, n = 20 [55.6%]; 5 µg, n = 18 [58.1%]) than placebo (n = 25 [73.5%]).

Conclusions: Our findings provide good initial evidence to suggest that tiotropium Respimat® may be administered with AeroChamber Plus® Flow-Vu® VHC in 1–5-year-old patients with persistent asthmatic symptoms. To confirm the clinical efficacy and safety in these patients, additional trials are required.

Clinical Trials Registry number: The trial was registered under NCT01634113 at http://www.clinicaltrials.gov.

Abbreviations: AE, adverse event; APSD, aerodynamic particle size distribution; DTL, dose-to-lung; EM, emitted mass; $fe_{0-3,ss}$, fraction of tiotropium excreted unchanged into urine between 0 and 3 h post-dose at steady state; FPD, fine particle dose; HPLC, high-performance liquid chromatography; ICS, inhaled corticosteroids; NGI, Next Generation Impactor; PK, pharmacokinetic; SIP, sample inlet port; VHC, valved holding chamber

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1. Introduction

Asthma is one of the most common chronic diseases in preschool children (≤5 years old) [1]. The preferred treatment in this age group is inhaled corticosteroids (ICS) [2]; however, limited information exists on treatment options for preschool children whose asthma symptoms are not well controlled with ICS [3]. A number of Phase 2 and 3 trials have demonstrated the efficacy and safety of tiotropium (administered via the Respimat® as an add-on therapy in adults, adolescents, and children > 5 years who suffer from persistent asthmatic symptoms) [4–11], warranting further investigation in younger children (≤5 years).

Correct handling of inhalers is essential for patients to achieve an adequate dose of prescribed medicine [12–14], but this can be problematic for young children, who often lack proper inhaler technique [13]. Therefore, the use of a valved holding chamber (VHC) with a face mask is recommended in young children [15].

The Respimat® is a handheld, propellant-free, multidose inhalation device that generates a slow-moving, long-lasting aerosol plume containing a large fine particle fraction (the fraction of dose containing particles < 5 μm) that enables efficient drug delivery to the lungs [16]. A previous *in vitro* study using throat models of children approximately 5 years old found that the use of the AeroChamber Plus® Flow-Vu® VHC for administering tiotropium Respimat® provided optimal dose-to-lung (DTL) in comparison with other VHCs, whilst minimizing retention at throat level [17]. Patients over the age of 5 years who are able to exhibit proper inhaler technique have been reported as having effective clinical outcomes following use of the Respimat® without a VHC [18]; moreover, children younger than 5 years can handle the Respimat® well when using a suitable VHC to complement its use [19].

A comprehensive assessment including *in vitro*, pharmacokinetic (PK), and clinical data is required when characterizing any inhalation product. Adequate characterization of the inhaler plus VHC with regards to particle distribution, drug availability, dosing, clinical safety, efficacy, and handling is therefore essential; however, *in vivo* determination of DTL in the paediatric population is challenging due to ethical restrictions on administering radiolabelled drugs to obtain scintigraphic pictures on lung deposition, as well as limited possibilities of using PK methods in young children. Blood sampling in young children is also ethically and practically difficult, but there is good correlation between *in vitro* DTL measurements and cumulative fractions of tiotropium in urine in children ≤5 years [20].

Here we present a combined *in vitro*, clinical, and PK characterization of tiotropium administration by Respimat® assisted by the AeroChamber Plus® Flow-Vu® anti-static VHC (hereafter referred to as test VHC) with face mask. We aimed to increase the scientific evidence for the administration of tiotropium Respimat® with the AeroChamber Plus® Flow-Vu® anti-static VHC in 1–5-year-olds with persistent asthmatic symptoms.

2. Methods

2.1. In vitro determinations

Depending on region, tiotropium Respimat® doses are available at two quantities (2.5 μg and 5 μg); we have used different dose strengths in individual data sets; all results are representative for both marketed doses, hereby referred to as "labelled dose".

2.2. Evaluation of aerodynamic particle size distribution under constant flow rates

2.2.1. Paediatric inhalation flow rates (constant flow rates) and face mask

We measured the aerodynamic particle size distribution (APSD) of tiotropium, administered by the Respimat® using a cascade impactor (Next Generation Impactor [NGI], Copley Scientific, Nottingham, UK). The Respimat® was tested on its own (as reference) or coupled to the VHC with an attached small- or medium-sized face mask (suitable for children aged ≤18 months and 1–5 years, respectively).

The paediatric flow rates (4.9 L/min, 8.0 L/min, and 12.0 L/min) were achieved by programming an electronic lung simulator (IngMar Medical, Pittsburgh, PA, USA) to generate a rectangular suction profile, consisting of the desired constant flow rate and a volume of 1 L. The start of the suction cycle was synchronized with the timer-controlled actuator of the Respimat® device. Delay times (the time between release of the dose and start of inhalation) were investigated by calculating the characteristic half-time periods (the time after which 50% of the aerosol remains available, whereas the other 50% remains deposited on the surface of the VHC). For the test VHC/face mask set-up, four delay times (0, 2, 5, and 10 s) were explored. A schematic representation of the experimental set-up is shown in Supplemental Fig. S1. The NGI was set to run at a constant flow rate of 30 L/min for all experiments. To translate the paediatric flow rates into the constant flow of 30 L/min through the NGI, a Mixing Inlet (Copley Scientific) was used. To simulate the high humidity in the lungs, the feed air was humidified (50% and 95% [± 5%] relative humidity for experiments with Respimat® alone; 50% for Respimat® with test VHC/face mask). To compare different ambient conditions, some experiments were also conducted using a refrigerated NGI (without humidification) to avoid evaporation of the droplets generated by Respimat®.

The total dose (emitted mass [EM] of % labelled dose) and APSD were quantified from depositions retained by the NGI using validated high-performance liquid chromatography (HPLC) methods. Sample separation inside the NGI is based on particle size, with impaction cup 1 trapping the largest particles and cup 7 retaining the smallest. For information on the cut-off diameter for each cup, please see Supplemental Table S1. The APSD was normalized to the labelled dose. The influence of the test VHC was evaluated by calculating ratios of fine particle dose (FPD; < 5 μm) and EM to the reference.

2.2.2. Determination of tiotropium particle size distribution achieved with the test VHC and mouthpiece when attached to the Respimat®, with and without delay

The Respimat® was connected to the test VHC and the face mask was removed. A timer-controlled shutter was actuated by a pneumatic cylinder. In the initial position, the shutter closed the test VHC and fed ambient air (22 °C, 50% relative humidity) into the sample inlet port (SIP). Activation of the shutter caused a driver connection between the test VHC and the SIP. Synchronization with release of the Respimat® was accomplished by a timer-controlled actuator, which was released before the shutter was opened after a defined delay time (0, 2, 5, and 10 s). The effect of three flow rates (30 L/min [primary flow rate]; 15 L/min, and 10 L/min [with no delay, included for information]) on APSD was measured using emissions from three individual test VHCs, with a total of 12 Respimat® devices. Delays were investigated using a single test VHC and four different Respimat® devices at the primary flow rate. The sampling time for all measurements was 6 s, resulting in evacuated volumes through the VHC of 3 L, 1.5 L, and 1 L for flow rates of 30 L/min, 15 L/min, and 10 L/min, respectively. For comparison with the reference (Respimat® alone), and as an estimate of the total dose emitted from the test VHC, the EM was calculated based on the mass captured by the impactor, including SIP and shutter.

2.3. Evaluation of EM under tidal breathing pattern (sinusoidal flow rates)

Breathing simulations were carried out as a representation of the functional interaction of valve and chamber volume with the aerosol cloud generated by the Respimat® device. An evaluation of EM by breathing simulation was investigated under the inclusion of the following factors: dose strength of labelled dose; test VHC with and without face mask (medium size); and fully coordinated and fully uncoordinated actuation. The breathing pattern was adjusted to the

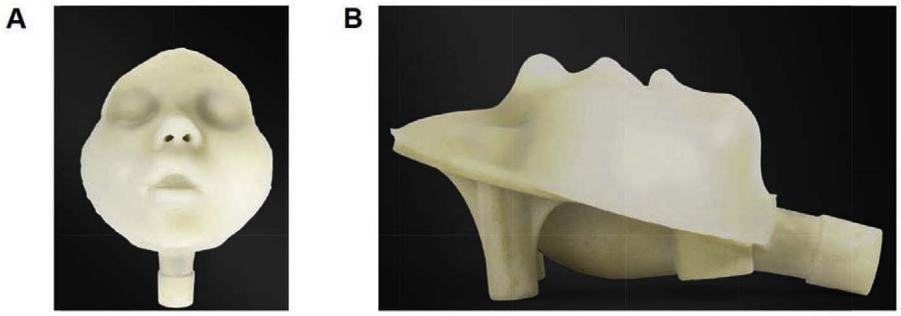

Fig. 1. The small child ADAM III face model; front view (**A**), side view (**B**).

intended age group of 1–5-year-olds, using a tidal volume of 155 mL, a frequency of 25 breaths per minute, a duty cycle of 33%, and a minute volume of 3875 mL. Configurations with and without face masks are shown in Supplemental Fig. S2. A sequence of either six actuations or 10 actuations, depending on dose strength per actuation, was scheduled. After each actuation, 10 breaths were simulated to guarantee complete emptying of the test VHC. A minimum of 30 s between the actuations was allowed for.

2.4. Comparison of Respimat® with and without test VHC using a child ADAM-III (face and upper airway) model and tidal breathing

To assess the mass of tiotropium likely to reach the lungs of a tidal-breathing 4-year-old child, an ADAM-III Child Model was used (Fig. 1). This model complements the experimental set-up described above by providing an anatomically correct upper airway, skeletal structure, and responsive facial features to measure delivered drug mass to the lung. The ADAM-III model was coated with surfactant (Brij), combined with the face and assembled with the test VHC, and then attached to the Respimat® and a medium-sized face mask. A force of ~16 N was applied to create the seal between the face mask and the model. The seal was checked by vacuum at 5.0 L/min. An ASL 5000 breathing simulator (IngMar Medical) was set up with a tidal volume of 155 mL, 25 breaths/minute, and an inspiratory-to-expiratory time ratio of 1:2; five actuations were delivered for each test. The collected drug was extracted by a water:acetonitrile (80%:20%) mixture at various specific positions in the model (including a filter at "carina" level), and assayed for active drug by HPLC ultraviolet spectrophotometry.

2.5. Clinical data

2.5.1. NinoTinA-asthma® study design, patient population, and study endpoints

Details of the study design and patient selection for NinoTinA-asthma® have been described elsewhere [21]. In brief, NinoTinA-asthma® was a randomized, double-blind, placebo-controlled, parallel-group, multicentre trial (NCT01634113) across 32 sites in 11 countries in Asia, Europe, and North America. Patients were randomized 1:1:1 to receive tiotropium 2.5 μg (two actuations of 1.25 μg), 5 μg (two actuations of 2.5 μg), or placebo, administered as two actuations via Respimat® once daily in the afternoon for 12 weeks, as add-on to usual maintenance therapy. Patients were included in the trial if they were aged 1–5 years, with at least a 6-month history of symptomatic persistent wheezing, cough, and/or shortness of breath, and with a need for ICS maintenance therapy to control asthma symptoms. Patients had to be able to correctly inhale from the Respimat® with or without VHC (AeroChamber Plus® Flow-Vu®). Children aged 5 years at the screening visit were permitted to use the Respimat® without VHC if they were able to demonstrate correct technique. All patients had to be on maintenance treatment with an ICS at stable dose, either as mono-treatment or in combination with another controller medication, for at least 4 weeks before screening. Patients had to be symptomatic (partly controlled), as defined by the Global Initiative for Asthma guidelines for children aged 5 years and younger, in the week prior to screening and in the week prior to randomization. Patients were excluded from the study if they had significant respiratory disease other than asthma, or any acute asthma exacerbation or respiratory tract infection within 4 weeks before screening.

The primary study objective was to evaluate the efficacy and safety of tiotropium. The co-primary efficacy endpoints were the weekly mean combined daytime asthma symptom score response (as assessed by the Paediatric Asthma Caregiver Diary in the last week), with a negative response indicating an improvement, and peak forced expiratory volume in 1 s from 0 to 3 h response (applicable only to children aged 5 years at screening who were able to perform a lung function test).

2.5.2. PK evaluation of tiotropium exposure in children vs. adults

Using a pooled analysis of nine Phase 2 and 3 trials (summarized in Table 1), we compared the systemic exposure to tiotropium administered by Respimat® in paediatric patients in NinoTinA-asthma® using the AeroChamber Plus® Flow-Vu® VHC versus systemic exposure in older children and adults (6–11 years old [1 trial]; 12–17 years old [1 trial]; ≥18 years old [7 trials]) with symptomatic persistent asthma not using a VHC. All patients aged 1–5 years in the PK subset used a VHC for the inhalation of tiotropium or placebo. Urine samples were collected from subsets of patients in individual trials following single and multiple dosing of tiotropium. Sampling for quantification of drug urine concentrations is described for each trial in Table 1.

Tiotropium concentrations in urine were determined by validated assays using HPLC coupled to tandem mass spectrometry [26] and analysed by Nuvisan Pharma Services GmbH & Co. KG (Neu-Ulm, Germany). The lower limit of quantification in urine was 10.0 pg/mL. Dose-normalized data (combined doses) were used to compare PK parameters obtained from adult patients with those from paediatric patients. Data were analysed using descriptive statistics.

3. Results

3.1. In vitro determinations

Different dose strengths were used in the individual data sets; results reported are representative for both marketed doses, referred to as "labelled dose".

3.2. Evaluation of APSD under constant flow rates

3.2.1. Paediatric inhalation flow rates (constant flow rates)

Young children generate different inhalation flow profiles in comparison with adults; to reflect this difference, three different paediatric inhalation flow rates (4.9 L/min, 8.0 L/min, and 12.0 L/min) were evaluated. These lower flow rates were selected to be representative of inspiratory flow rates of children aged 6–12 months, 2–5 years, and > 5 years, respectively. In cases where low ("paediatric") flow rates were used, the transmission of FPD by the Respimat® when attached to the

Table 1
Summary of trial designs of studies included for PK evaluation.

Patient groups	Trials	Asthma severity	Design[a]	Duration	Maintenance therapy	Treatment doses[bc]	Pre-dose urine sampling time points (hours)	N in PK analysis
1–5 years	205.443 NinoTinA-asthma® (NCT01634113)	Persistent	Phase 2/3, parallel group	12 weeks	Stable-dose ICS, alone or in combination with another controller medication	TioR 5 TioR 2.5 PboR	−1 − 0, 0–2, 2 − 3	3 12 9
6–11 years	205.425 [4] (NCT01383499)	Moderate	Phase 2, incomplete crossover	12 weeks (3 × 4 weeks)	ICS (200–400 μg budesonide or equivalent dose) with or without an LTRA	TioR 5 TioR 2.5 TioR 1.25 PboR	−1 − 0, 0–3, 3 − 24	12 12 11 15
12–17 years	205.424 [5,22] (NCT01122680)	Moderate	Phase 2, incomplete crossover	12 weeks (3 × 4 weeks)	ICS (200–800 μg budesonide or equivalent dose) with or without an LTRA	TioR 5 TioR 2.5 TioR 1.25 PboR	−1 − 0, 0–3, 3 − 24	14 13 14 11
> 18 years (range 18–75 years)	205.380 [22,23] (NCT01233284)	Moderate	Phase 2, crossover	16 weeks (4 × 4 weeks)	ICS (400–800 μg budesonide or equivalent dose)	TioR 5 TioR 2.5 TioR 1.25 PboR	−1 − 0, 0–3, 3 − 24	49 51 52 51
	205.420 [22,24] (NCT01152450)	Moderate	Phase 2, crossover	12 weeks (3 × 4 weeks)	ICS (400–800 μg budesonide or equivalent dose)	TioR 5 TioR 2.5 PboR	−1 − 0, 0–6, 6–12, 12 − 24	28 29 29
	205.441 [22,25] (NCT01696071)	Moderate	Phase 2, two-way crossover	8 weeks (2 × 4 weeks)	ICS (400–800 μg budesonide or equivalent dose)	TioR 5 TioR 2.5	−1 − 0, 0–6, 6–12, 12 − 24	28 29
	205.416/205.417 PrimoTinA-asthma® [7,22] (NCT00772538/ NCT00776984)	Severe	Two replicate Phase 3, parallel group	48 weeks	ICS (≥800 μg budesonide or equivalent dose) with a LABA	TioR 5 PboR	−1 − 0, 0–2, 2–6, 6 − 24	37/38 34/38
	205.418/205.419 MezzoTinA-asthma® [8,22] (NCT01172808/ NCT01172821)	Moderate	Two replicate Phase 3, parallel group, active comparator	24 weeks	ICS (400–800 μg budesonide or equivalent dose)	TioR 5[d] TioR 2.5[d] Salmeterol 50[e] Placebo[f]	−1 − 0, 0–2, 2–6, 6 − 24	35/23 33/28 36/26 36/23

Abbreviations: HFA-MDI, hydrofluoroalkane metered-dose inhaler; ICS, inhaled corticosteroids; LABA, long-acting β2-agonist; LTRA, leukotriene receptor antagonist; PboR, placebo delivered via the Respimat® Soft Mist™ Inhaler; PK, pharmacokinetic; TioR 1.25, TioR 2.5, and TioR 5, tiotropium 1.25 μg, 2.5 μg, and 5 μg, respectively, delivered via the Respimat® Soft Mist™ Inhaler.

[a] All trials conducted in a randomized, double-blind, and placebo-controlled manner with the exception of 205.441 (NCT01696071), which was not placebo-controlled.

[b] Administered once daily via the Respimat® Soft Mist™ Inhaler unless otherwise stated.

[c] All treatments as add-on to usual maintenance therapy.

[d] Plus placebo administered twice daily (evening and morning) via HFA-MDI.

[e] 50 μg administered twice daily (evening and morning) via HFA-MDI plus PboR (evening).

[f] Double-dummy placebo administered once daily (evening) via the Respimat® Soft Mist™ Inhaler and twice daily (evening and morning) via HFA-MDI.

Table 2
Ratios of fine particle dose (< 5 μm) and delivered dose with and without VHC.[a]

	FPD, < 5 μm; % labelled dose			Delivered dose, % labelled dose		
Flow rate	Respimat® with VHC	Respimat® only	**Ratio**	Respimat® with VHC	Respimat® only	**Ratio**
4.9 L/min	28	38	0.7	34	95	0.4
8.0 L/min	27	46	0.6	30	97	0.3
12.0 L/min	36	50	0.7	46	98	0.5
30 L/min[b]	51	63	0.8	79	96	0.8

Abbreviations: FPD, fine particle dose; VHC, valved holding chamber.

[a] At 23 °C, 50% relative humidity, and no delay using different paediatric flow rates.

[b] Adult flow rate is included for comparison.

test VHC/face mask was approximately 60–70% of that generated by the Respimat® alone (Table 2) compared with 80% with the higher flow rate of 30 L/min (representative of inspiratory flow rate of adults), which resulted in less than 20% loss. Compared with adult flow rates with Respimat® alone, the FPD transmitted with paediatric flow rates by Respimat® and VHC was approximately 50% less. The ratio of delivered dose by the Respimat® plus test VHC/face mask compared with the Respimat® alone ranged between 30% and 50% for low ("paediatric") flow rates (Table 2), indicating a lower transmission with the test VHC for all particles generated by the Respimat®. In combination with the FPD transmission ratio, these results suggest that coarse particles are preferentially captured by the test VHC, whereas fine particles stay available to the patient. Since the purpose of the test VHC is to retain larger droplets and particles, these results were as expected.

The half-time period (the time after which 50% of the aerosol remains available) values for the decrease of aerosol in the test VHC were 17.8 s, 19.3 s, and 11.2 s at flow rates of 4.9 L/min, 8.0 L/min, and 12.0 L/min, respectively. Corresponding half-time periods for FPD were 17.8 s, 16.1 s, and 10.7 s for flow rates of 4.9 L/min, 8.0 L/min, and 12.0 L/min, respectively. These results show that faster flow conditions considerably shortened the half-time period and decreased the amount of aerosol recovered from the test VHC, indicating that either impaction

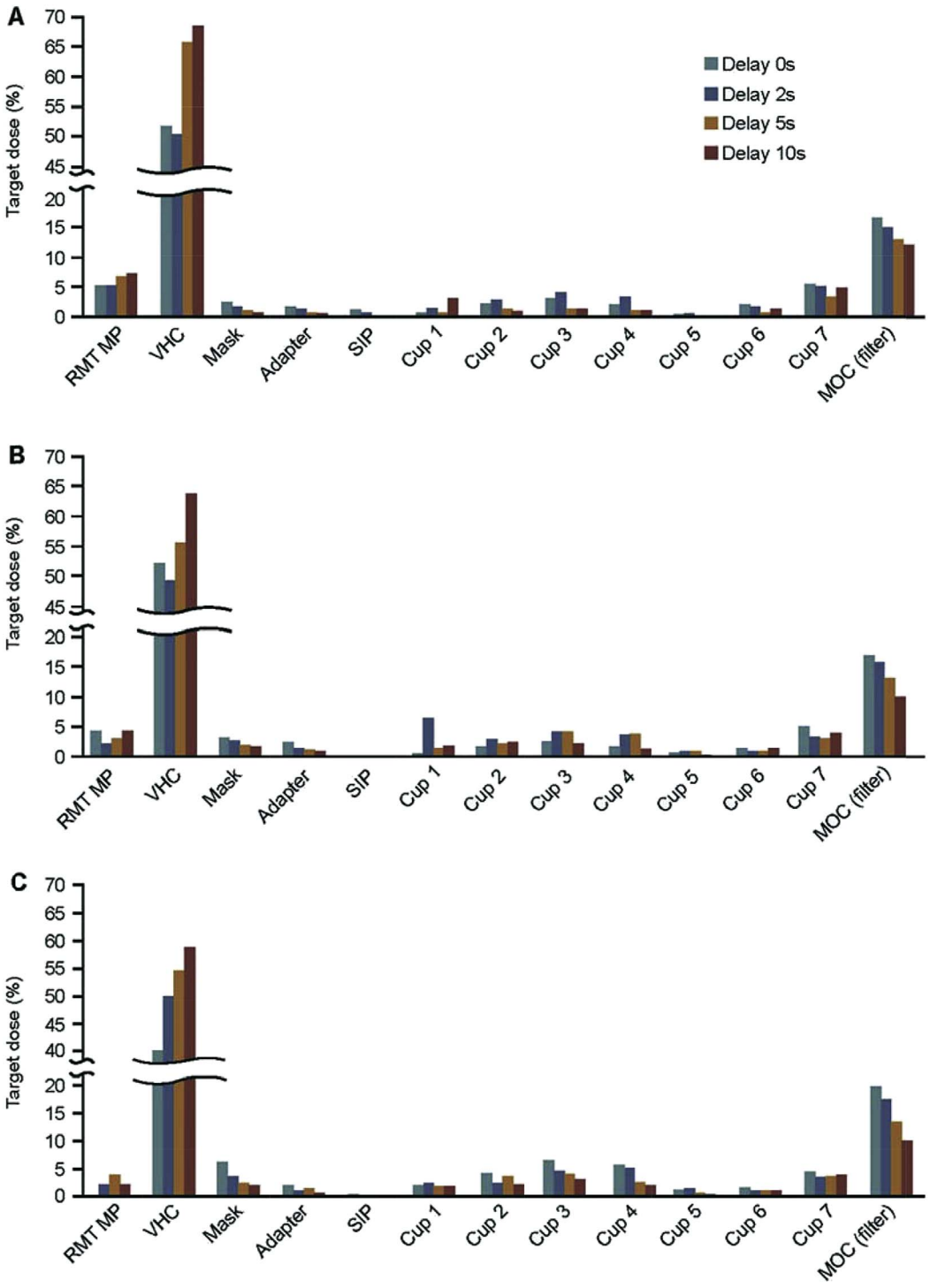

Fig. 2. Particle size distributions in different compartments by paediatric flow rate and delay time.*
*Respimat® plus small-/medium-sized face mask; 4.9 L/min (**A**), 8.0 L/min (**B**), and 12.0 L/min (**C**).
Abbreviations: MOC, multi-orifice collector; RMT MP, Respimat® mouthpiece; SIP, sample inlet port; VHC, valved holding chamber.

in the outlet region of the chamber played an important role, or that the air flowing into the chamber may give rise to increased aerosol deposition in the chamber.

The fine particle fraction of the delivered dose from the test VHC was 69–89%, which was more than that which was typically delivered by the Respimat® alone (60%), indicating that coarse particles are preferentially retained by the VHC. The full particle-size distributions for all paediatric flow rates and all delays are displayed in Fig. 2.

A summary of the *in vitro* data for delivered dose at the different flow rates and holding times is shown in Table 3. In terms of dose per kg/body weight, *in vitro*-delivered dosing at flow rates corresponding to preschool children was comparable with that at flow rates corresponding to older children. A holding time of 0 s means that the air flow is already swirling through the air vents when the dose is released. At a holding time of 2 s, highly turbulent air flow around the nozzle spray generation is avoided, leading to reduced deposition inside the Respimat® mouthpiece and/or the test VHC. At higher flow rate, the effect of emptying the chamber gains the highest importance.

Table 3
In vitro medication delivery through the test VHC plus small- or medium-sized face mask.

Flow rate, L/min, 50% RH and corresponding age[a]	Mask	Holding time, s	Mean medication delivery through test VHC, μg/dose (± SD)	Body weight 50th percentile, kg[b]	Medication deliveredper dose, ng/kg[c]
4.9 L/min	Small	0	0.85 (± 0.04)	7.5–9.9	86–113
(6–12 months)		2	0.86 (± 0.14)		87–115
		5	0.55 (± 0.16)		56–73
		10	0.62 (± 0.02)		63–83
8.0 L/min	Medium	0	0.74 (± 0.05)	12.3–18.0	41–60
(2–5 years)		2	0.93 (± 0.05)		52–76
		5	0.72 (± 0.07)		40–59
		10	0.57 (± 0.05)		32–46
12.0 L/min	Medium	0	1.16 (± 0.07)	18.0	64
(> 5 years)		2	0.96 (± 0.0)		53
		5	0.78 (± 0.18)		43
		10	0.61 (± 0.02)		34

Abbreviations: RH, relative humidity; SD, standard deviation; VHC, valved holding chamber.
[a] Data corresponding to age group 12–23 months are not available.
[b] Centers for Disease Control growth charts, developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion. Body weight values correspond to the average of the 50th percentile weight for boys and girls at the ages indicated.
[c] Inhalation of 2.5 μg tiotropium by Respimat® (as two actuations) in a 70 kg adult not using a VHC and mask delivers approximately 2.5 μg or 36 ng/kg.

3.2.2. Determination of tiotropium particle size distribution achieved with the test VHC and mouthpiece when attached to the Respimat®, with and without delay

Inertial impaction, gravitational sedimentation, and electrostatic attraction with the VHC wall chamber can all impact on aerosol particles passing through a VHC. These factors can be accentuated by time delays between actuation and inhalation [27]. We therefore determined the effect of delayed inspiration after actuation of tiotropium Respimat®. Due to the proportional increase in delivered dose between the two dose strengths (within experimental error), the results are reported as average of both strengths, expressed in percentage of labelled dose.

APSD emitted by the Respimat® when attached to the test VHC with a mouthpiece was measured with and without delay in a second, alternative set-up containing a shutter (United States Pharmacopeial Convention draft chapter 1602) [28]. For reference, emission by Respimat® alone (without the test VHC) was also determined. The delivered dose with the Respimat® alone was close to 100% of the labelled dose. The cumulative particle size distributions are shown in Supplemental Fig. S3. Flow rate dependence and influence of delay between actuation and start are shown in Fig. 3. The output of the test VHC was found to depend on the flow rate; EM and FPD decreased with decreasing flow rate. As expected, delays resulted in a more pronounced sedimentation of tiotropium, since the aerosol stays longer in the VHC, resulting in a lower amount of aerosol available to patients.

At 50% relative humidity, the ratios of the FPD at the exit of the test VHC and the reference FPD value of the Respimat® alone were 70% for the flow rate of 15 L/min and 80% for 30 L/min. These high ratios indicate a low loss of fine particles, thus corresponding to good transmission properties of the test VHC. EM ratios of test VHC:Respimat® reference were 60% and 80% for 15 L/min and 30 L/min, respectively, indicating improved transmission properties of the test VHC at higher flow rates, with preferential entrapment of larger particles (> 5 μm) in the test VHC at lower flow rates.

3.2.3. Evaluation of EM under tidal breathing pattern (sinusoidal flow profiles)

We included simulations of tidal breathing, as this type of breathing is the most commonly encountered breathing pattern in preschool children who cannot master a forced inhalation manoeuvre.

The standardized tidal inhalation of a child resulted in an EM from the VHC that was approximately one-third of the labelled dose. This finding was independent of coordination (fully coordinated *vs.* fully uncoordinated) and of the use of a face mask. The mass balance (total tiotropium recovered from the mouthpiece, adapter, test VHC, and EM from the test VHC and face mask, where used) was > 90%, indicating a sufficient recovery. Averages for EM at the exit of the test VHC by breathing simulation, drug retained in the test VHC during breathing simulation, and drug retained in the face mask during breathing simulation (all expressed as percentage of labelled dose) are shown in Table 4.

3.2.4. Evaluation of the Respimat® with VHC using a child ADAM-III model

The ADAM-III model was included to complement the experimental set-up by adding an anatomically correct upper airway, skeletal structure, and responsive facial features to measure delivered drug mass to the lung. Approximately 10% of tiotropium emitted by the Respimat® was recovered from the model face and upper airway, 22% was retained by the filter (corresponding to the carina, indicating the amount of tiotropium available to the lungs), and 49% was retained in the VHC and face mask. These data correlated well with results from the tidal breathing simulation described above (Table 5), whilst adding additional information about the amount of drug reaching the carina level of the airways.

3.3. PK evaluation

Baseline demographics of the PK populations, separated into children aged ≤5 years, children 6–11 years old, adolescents 12–17 years old, and adults ≥18 years, are shown in Table 6. Twelve patients had normal renal function (estimated glomerular filtration rate based on serum creatinine > 90 mL/min/1.73 m^2) and three patients had mild renal impairment (estimated glomerular filtration rate values based on serum creatinine in the range of 60–90 mL/min/1.73 m^2). The fraction of tiotropium excreted unchanged into urine between 0 and 3 h post-dose at steady state ($fe_{0–3,ss}$) was 52–60% lower in 1–5-year-old patients compared with older children and adults (Table 7). Following normalization of $fe_{0–3,ss}$ by body size parameters such as height or body surface area (to account for the smaller lung volume in younger patients), total tiotropium exposure was comparable between the different age groups (Fig. 4).

3.4. Clinical data

3.4.1. NinoTinA-asthma® efficacy and safety

Detailed results of NinoTinA-asthma® have been published elsewhere [21]. In brief, 102 of the enrolled 129 patients were randomized to either tiotropium 2.5 μg (n = 36), tiotropium 5 μg (n = 32), or placebo (n = 34). Overall, 101 patients were treated and completed the

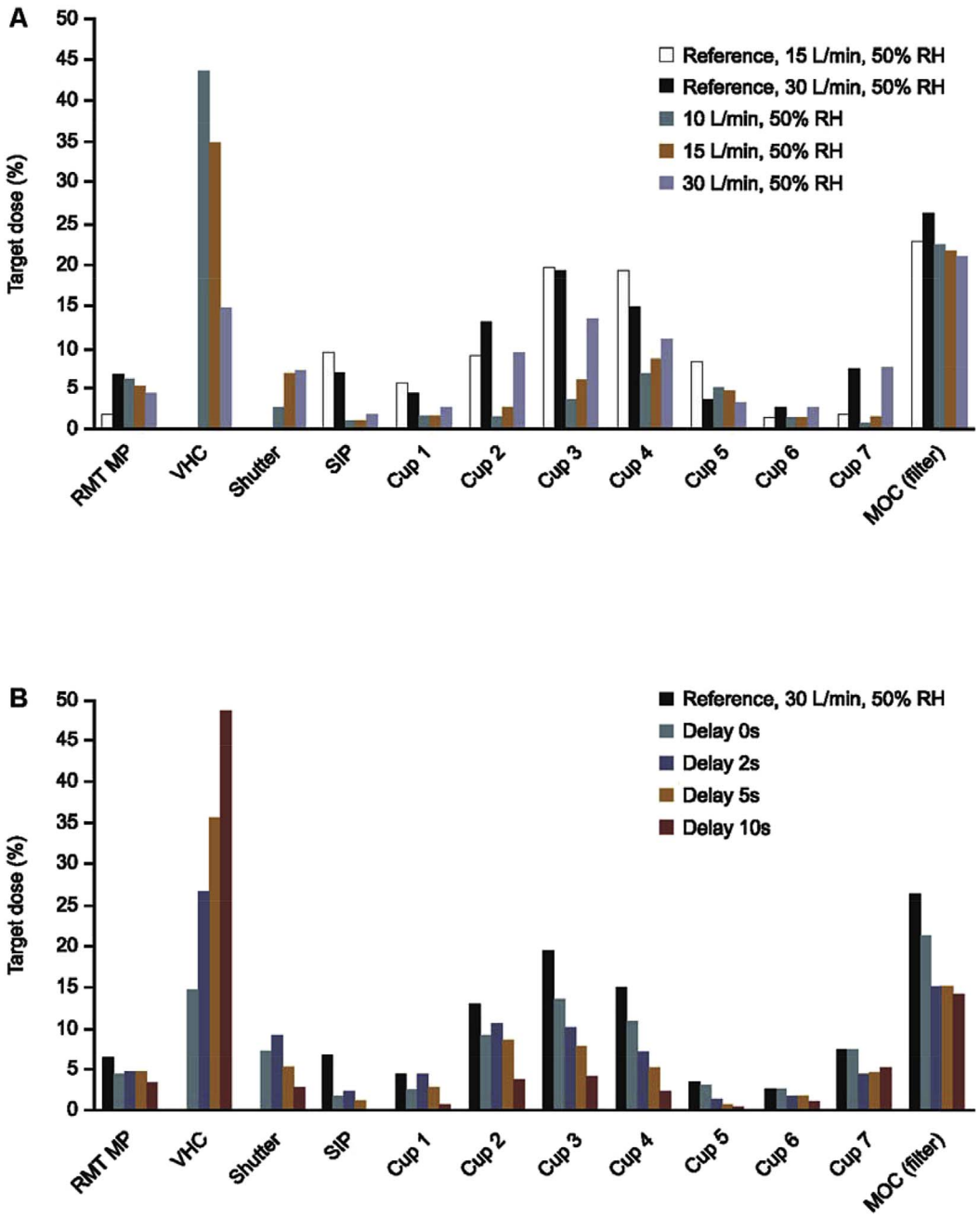

Fig. 3. Flow rate dependence of APSD (**A**); influence of delay between actuation and inhalation start (**B**).*
*In comparison with reference baseline results (Respimat® only; displayed as white [15 L/min at 50% relative humidity] and black-and-white [30 L/min at 50% relative humidity] bars).
Abbreviations: APSD, aerodynamic particle size distribution; MOC, multi-orifice collector; RH, relative humidity; RMT MP, Respimat® mouthpiece; SIP, sample inlet port; VHC, valved holding chamber.

12-week treatment period. All but one patient per treatment group used the VHC. Fewer patients in the tiotropium groups reported any adverse events (AEs) compared with the placebo group (tiotropium 2.5 µg, 55.6%; tiotropium 5 µg, 58.1%; placebo, 73.5%). No AEs led to discontinuation or death; serious AEs were reported in three patients (placebo only). Asthma exacerbations (16.8%), nasopharyngitis (13.9%), and pyrexia (11.9%) were reported in > 10% of the patients in total. Generally, frequencies of AEs were balanced across treatment groups.

Improvement in weekly mean combined daytime asthma symptom score response (co-primary endpoint; as determined by Paediatric Asthma Caregiver Diary) was observed in all treatment groups. Only a small number of patients provided lung function data ($n = 4$ in placebo group; $n = 7$ in tiotropium 2.5 µg group; $n = 2$ in tiotropium 5 µg group); no meaningful interpretations of the results could be reached.

Asthma exacerbations, reported as AEs, were reported by a higher proportion of patients in the placebo group (29.4%) than in the tiotropium arms (2.5 µg, 13.9%; 5 µg, 6.5%).

4. Discussion

Herein, we present complementary *in vitro*, clinical, and PK evidence for the administration of tiotropium Respimat® using the AeroChamber Plus® Flow-Vu® VHC in 1–5-year-old patients with persistent asthmatic symptoms.

The correct use of an inhaler is imperative to achieve an appropriate dose delivered to the lungs. The Respimat®, which is currently the only commercially available soft mist inhaler, contains a mechanical spring

Table 4
Averages for emitted mass at the exit of the VHC.[a]

Operation	Emitted mass, % (SD)	Drug retained in VHC, % (SD)	Drug retained in face mask, % (SD)
Fully coordinated, no face mask	36 (5)	34 (6)	N/A
Fully uncoordinated, no face mask	35 (6)	34 (5)	N/A
Fully coordinated, with face mask	32 (5)	34 (3)	11 (2)
Fully uncoordinated, with face mask	30 (4)	38 (5)	11 (1)

Abbreviations: N/A, not available; SD, standard deviation; VHC, valved holding chamber.
[a] By breathing simulation, drug retained in the VHC during breathing simulation, and drug retained in the face mask during breathing simulation (all expressed as % of labelled dose).

Table 5
Comparison of tiotropium retained by components of the ADAM-III face model compared with breathing simulations.[a]

	ADAM-III model, % of labelled dose retained	Breathing simulation, % of labelled dose retained[b]	
		Fully uncoordinated	Fully coordinated
Face	2.2	N/A	N/A
Airways	6.4		
Filter	22.1	30	32
Sum face-to-filter[c]	30.7	30	32
VHC	44.5	38	34
Mask	4.5	11	11
Total mass delivered	79.7	79	77

Abbreviations: N/A, not available; VHC, valved holding chamber; V_t, tidal volume.
[a] V_t = 155 mL.
[b] With face mask.
[c] As incorporated by child.

that atomizes the drug solution, creating a fine, slow-moving mist with a relatively higher deposition in the lung, and less drug deposited in the mouth and throat [29]. A recent handling study of the Respimat® in children under 5 years old found that all children using the AeroChamber Plus® Flow-Vu® VHC and face mask, either by themselves or with adult assistance, achieved successful handling, and thus recommended that a VHC should be used to administer tiotropium via the Respimat® in children younger than 5 years old [19]. In addition to ensuring correct handling of the device, there is also a need for safety and optimal clinical control of aerosol delivery in children, and for a realistic prediction of the dose delivered to the lung. In the NinoTinA-asthma® trial, in preschool children with persistent asthmatic symptoms, the AeroChamber Plus® Flow-Vu® was chosen as the test VHC because it has previously been shown to support optimal DTL by the Respimat® in comparison with other VHCs, whilst minimizing retention

Table 7
Amount of tiotropium excreted unchanged in urine over 3 h post-dose by age group.

Age category	$fe_{0-3,ss}$	
	N[a]	gMean (gCV %)
1–5 years	15	1.16 (59.7)
6–11 years	31	2.43 (69.4)
12–17 years	32	2.92 (85.8)
≥18 years	147	2.52 (107)

Abbreviations: fe_{0-3ss}, fraction of dose excreted unchanged into urine between 0 and 3 h post-dose at steady state; gCV, geometric coefficient of variation; gMean, geometric mean.
[a] Some patients contributed observations more than once in the case of crossover study design.

at throat level [17]. We evaluated the dose delivered by the Respimat® with the test VHC in young children by carrying out *in vitro* studies – as well as by comparing the systemic exposure with tiotropium in preschool patients in NinoTinA-asthma® using the AeroChamber Plus® Flow-Vu® VHC, and older children (6–17 years old) and adults (≥18 years old) with symptomatic persistent asthma not using VHC – in a pooled analysis of nine Phase 2 and 3 trials. The safety of tiotropium administered via the Respimat® inhaler and the test VHC was comparable with placebo, and tiotropium showed a potential to reduce asthma exacerbation risk compared with placebo. However, to confirm the findings, additional clinical trials are required.

In our three-part *in vitro* study, we first carried out a comprehensive characterization of the tiotropium dose passing through the AeroChamber Plus® Flow-Vu® VHC by evaluating the tiotropium particle size distribution delivered into a cascade impactor. We assessed fixed inspiratory flow rates, including those representative of children aged 6–12 months, 2–5 years, and > 5 years. We found that the output of the VHC depended on the flow rate, and that EM and FPD decreased with decreasing flow rate. A dose reduction was observed when Respimat® was administered by test VHC at low ("paediatric") flow rates compared with Respimat® dosing without VHC/face mask using flow rates representative of adults. In terms of dose per kg/body weight, we found that under all tested conditions, the Respimat®, when used with the test VHC and mask *in vitro*, delivered dosing comparable to with that of adults without the use of the test VHC and face mask (Table 3); this was expected given the allometric scaling. When used with the test VHC, the transmission and holding properties of the Respimat® were found to be fully sufficient for the delivery of aerosol to patients. At no delay, the most relevant FPD (< 5 µm) exhibited a transfer efficacy of ≥60%. The half-time of the FPD was > 10 s, giving enough time to allow multiple breaths.

Cascade impactors are designed to be operated at fixed flow rates, and cannot be used to monitor the operation of critical moving components, such as the inhalation and exhalation valves of VHCs. We therefore included a second set of experiments that simulated tidal breathing, as this type of respiratory pattern is most commonly encountered with patients using a VHC, particularly small children who are incapable of a forced inhalation manoeuvre. We found that the standardized tidal inhalation of a child results in an EM from the test VHC that is approximately one-third of the labelled dose. This value

Table 6
Summary of baseline patient demographics in the PK subsets.

	1–5 years (*n* = 15)	6–11 years (*n* = 31)	12–17 years (*n* = 32)	≥18 years (*n* = 52)
Age, years, median (min, max)	4.0 (1.0, 5.0)	9.0 (6.0, 11.0)	14.0 (12.0, 17.0)	49.0 (22.0, 75.0)
Body weight, kg, median (min, max)	18.0 (12.0, 40.0)	34.3 (23.0, 56.0)	54.0 (35.0, 115.0)	79.0 (56.0, 110.8)
Height, cm, median (min, max)	111.0 (77.0, 126.0)	143.0 (116.0, 162.0)	165.5 (146.0, 185.0)	170.0 (150.0, 191.0)
Body surface area, m^2, median (min, max)	0.8 (0.5, 1.1)	1.2 (0.9, 1.6)	1.6 (1.2, 2.4)	1.9 (1.6, 2.2)
Male, n (%)	8.0 (53.3)	21.0 (67.7)	23.0 (71.9)	20.0 (38.5)

Abbreviation: PK, pharmacokinetic.

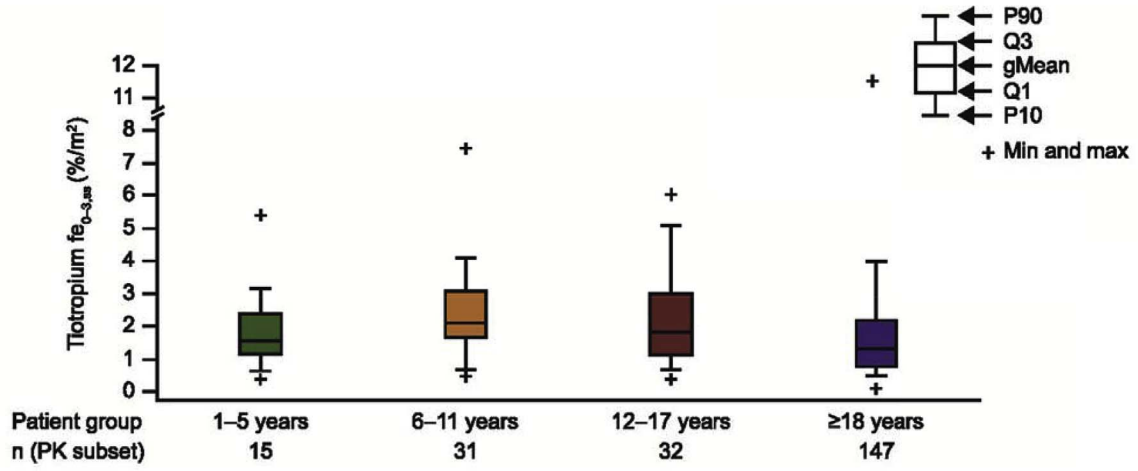

Fig. 4. $Fe_{0–3,ss}$ values following multiple-dose administration of tiotropium to steady state by age group.*
*Corrected by body surface area.
Abbreviations: $fe_{0–3ss}$, fraction of dose excreted unchanged into urine between 0 and 3 h post-dose at steady state; gMean, geometric mean; PK, pharmacokinetic.

was independent of coordination (fully coordinated *vs.* fully uncoordinated) and of the use of a face mask, indicating predictable tiotropium administration by the Respimat®. Our results correspond well with a previous study of tidal breathing simulations in an idealized throat model of a child of approximately 5 years of age, which found a DTL of 33% of the label claim when the Respimat® was administered with the test VHC [17].

To realistically mimic the conditions of patient use, a representation of a patient face needs to be included in the test set-up, so that the interface between face mask and aerosol measurement apparatus can be realized. The ADAM-III model used in this study is based on the anatomy of a 4-year-old, 15.9 kg male child, and offers possibility for oral, oral and nasal, and obligate nasal breathing [30]. Furthermore, an anatomically correct upper airway is incorporated into the ADAM-III model, allowing for the delivered mass to be estimated at the carina level. The inclusion of modelled soft tissue is of critical importance, as it simulates where the mask "lands" on the face when a clinically appropriate force is employed. The soft tissue of the model needs to respond to the mechanical force when the face mask is applied to simulate the dead space between face mask and face, and allows for possible leakage between the lip of the face mask and face to be evaluated [31]. When including the ADAM-III model, we found that 80% of the mass emitted from the Respimat® was delivered to the filter, device, or mask, with 20% being retained on the model face and airways. This compares well with the 78% of EM retained in our tidal breathing simulation, confirming the findings under more physiologically relevant conditions.

In vitro determinations of delivered DTL with the Respimat® and this VHC serve as a good predictor of *in vivo* tiotropium delivery to the lungs of children. Using mouth–throat models and real-life inhalation profiles of children aged 0–5 years, Bickmann et al. [20] calculated that the DTL from the Respimat® plus VHC ranged from 5.1% to 37.1%, depending on the age of the child. The calculated *in vitro* DTL per body mass ranged between 0.031 and 0.066 μg/kg, which compared well with that of adults (0.046 μg/kg), suggesting that despite the seemingly low percentages the efficacy of the treatment was not negatively impacted. Using tidal breathing conditions and the ADAM-III model, we calculated that over 30% of emitted tiotropium is expected to reach the lungs of a young child when the test VHC is used.

Our *in vitro* findings are confirmed by the results of the PK studies, in which exposure to tiotropium was comparable between age groups when normalized for body surface area, suggesting that children in the NinoTinA-asthma® trial were not overexposed to tiotropium [21]. These findings support the safety evaluation in this age group, and also indicate adequacy of both exposure to tiotropium and dosing. Total tiotropium exposure in patients aged 1–5 years was 52–60% lower compared with that in the other age groups. Given that normal renal function was observed in most of the preschool patients, this lower exposure to tiotropium could have been due to the use of the VHC and face mask for inhalation; a reduction in the amount of drug delivered due to the use of a VHC has been documented previously [20].

We are the first to provide a comprehensive series of *in vitro* and clinical investigations to support the administration of tiotropium Respimat® assisted by the AeroChamber Plus® Flow-Vu® VHC in preschool children, and our *in vitro* models complement our clinical findings. We accept as a possible limitation of our study that only one type of VHC was tested; however, VHCs are not interchangeable, and appropriate clinical metrics are needed to assess the efficacy and safety of any inhalation product. As such, data from appropriate clinical trials must be obtained [20].

5. Conclusion

In conclusion, our *in vitro*, PK, and clinical results present increased scientific evidence for the administration of tiotropium Respimat® with AeroChamber Plus® Flow-Vu® VHC in 1–5-year-old patients with persistent asthmatic symptoms. For the full evaluation of clinical efficacy and safety in these patients, additional clinical trials are required.

Declaration of interest

Herbert Wachtel is an employee of Boehringer Ingelheim and has a patent (WO2004024340A1-Blockiervorrichtung für ein Sperrspannwerk) with royalties paid according to German law regulating inventions by employees. Georges El Azzi, Michael Engel, and Ashish Sharma are employees of Boehringer Ingelheim. Mark Nagel and Jason Suggett are employees of Trudell Medical International. Herbert Wachtel is co-inventor of parts of the Respimat® inhaler.

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Author contributions

Herbert Wachtel proposed the *in vitro* study design, and contributed to the generation and interpretation of the *in vitro* data. He also contributed to the drafting of the manuscript. Jason Suggett and Mark

Nagel contributed to the generation of some study data, interpretation of *in vitro* data, and drafting of the manuscript. Michael Engel was involved in devising the study concept and design of the NinoTinA-asthma® trial, in carrying out the study programme and data analysis, and in the preparation and review of the manuscript. Georges El Azzi was involved in study conduct, data analysis, and in the preparation and review of the manuscript. Ashish Sharma was involved in study design, analysis, interpretation, and reporting.

Ethics approval and consent to participate

Written informed consent was obtained from all patients enrolled in the clinical studies. All studies were approved by ethical committees and registered on clinicaltrials.gov. For the NinoTinA-asthma® study reported here, before the start of the study, the clinical trial protocol, patient information leaflet, informed consent form, and other locally required documents were reviewed by the Independent Ethics Committees or Institutional Review Boards, or both, of the participating centers. Subsequently, the Centrale Commissie Mensgebonden Onderzoek (The Hague, Netherlands) approved the trial.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2018.03.010.

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