SYNOPSIS CLINICAL STUDY REPORT

according to ICH E3 guideline

Version 1.0F, 25.06.2025

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSS-OVER STUDY TO EVALUATE THE EFFICACY AND SAFETY OF TIOTROPIUM INHALATION SOLUTION (5 µg) VIA RESPIMAT® INHALER ONCE DAILY FOR 24 WEEKS IN CHILDREN (6 TO 12 YEARS) WITH BRONCHOPULMONARY DYSPLASIA

TRIBOR-STUDY

Trial Protocol version 3.0F, 06.11.2020 including amendment 01 version 4.0F, 16.09.2022, amendment 02 version 5.0F, 30.03.2023

Sponsor	**Technische Universität Dresden** 01062 Dresden
Principal Coordinating Investigator	**Prof. Dr. Christian Vogelberg** Medizinische Fakultät der TU Dresden Klinik und Poliklinik für Kinder- und Jugendmedizin Fachbereich Kinderpneumologie, Allergologie Fetscherstraße 74, 01307 Dresden
Sponsor Code:	TUD-TRIBOR-072
EudraCT-Number:	2020-000529-19
Study number of Boehringer Ingelheim	0205-0544
Name of Finished Product and Active Substance	Finished Product: Spiriva Respimat® Active Substance: Tiotropiumbromid Finished Product: Placebo Active Substance: not applicable

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1 SUMMARY OF TRIAL INFORMATION

Sponsor	Technische Universität Dresden 01062 Dresden
Principal Coordinating Investigator	Prof. Dr. Christian Vogelberg
Full Title	A randomized, double-blind, placebo-controlled, cross-over study to evaluate the efficacy and safety of tiotropium inhalation solution (5 µg) via Respimat® inhaler once daily for 24 weeks in children (6 to 12 years) with bronchopulmonary dysplasia
Short Title	TRIBOR-Study
Trial Protocol	Trial Protocol version 3.0 F, 06.11.2020 including amendment 01 version 4.0 F, 16.09.2022, amendment 02 version 5.0 F, 30.03.2023
Indication	Bronchopulmonary dysplasia
Phase of development	Phase II
Study design	Randomized, double-blind, placebo-controlled, cross-over
Objective(s) of the clinical trial	Primary objective(s): Investigation of the clinical efficacy on lung function (FEV_1 peak (0-1h)) of a daily inhalation of 5 µg tiotropium via Respimat® inhaler for twelve weeks in 6-12-year-old children with bronchopulmonary dysplasia Secondary objectives: To investigate the safety and efficacy of daily inhalation of 5 µg tiotropium via Respimat® inhaler in 6-12-year-old children with bronchopulmonary dysplasia on various lung function parameters and physical activity as measured by the Physical Activity Questionnaire for Children (PAQ-C) in German translation
Endpoints of the clinical trial	Primary Endpoint(s): Change in FEV_1 peak (0-1 h) after 12 weeks of treatment Secondary Endpoints of Efficacy: Change in the following parameters after 12 weeks of treatment: FEV_1 before inhalation FEV_1 AUC (0- 1h) FVC peak (0-1h) FVC before inhalation FVC AUC(0-1h) FEV_1/FVC (Tiffeneau-Pinelli index) before inhalation MEF_{75-25} before inhalation difference of FEV_1, FVC, and MEF_{75-25} before and after standardized treadmill provocation

	PEF am/pm PEF variability Physical Activity Questionnaire for Children (PAQ-C) <u>Secondary Endpoints of Safety:</u> AEs and SAEs
Number of patients	planned sample size: 26 patients incl. 20% drop out patients screened: 100 patients enrolled: 23 patients patients analysed: 23 patients
Studied period	First patient in: July 7, 2021 Last patient in: December 13, 2023 Last patient last visit: June 26, 2024 On April 5th, 2024, recruitment was prematurely stopped after enrolment of 23 patients instead of 26 patients due to slow recruitment rate. The planned number of evaluable patients was reached.
Inclusion criteria	1. Presence of bronchopulmonary dysplasia 2. Former premature male and female patients aged 6 to 12 years 3. Written informed consent of patient and its legal guardians 4. FEV_1 before inhalation of a betamimetic >60% and <90% of target 5. Positive bronchial reversibility test, i.e. increase in FEV_1 by ≥12% 15-30 min after inhalation of 200 µg salbutamol 6. Ability to operate the Respimat® inhaler correctly 7. Ability to perform the study-related examinations
Exclusion criteria	1. History of known hypersensitivity to tiotropium or its ingredients or to drugs with a similar chemical structure 2. Participation of the patient in another clinical trial within the last 4 weeks prior to inclusion 3. Indications that the patient is unlikely to comply with the protocol (e.g. unwillingness to cooperate) 4. Contraindications according to the Spiriva® Respimat® Information for healthcare professionals 5. Presence of bronchial asthma 6. Presence of allergic sensitization to typical regional respiratory allergens 7. Therapy with inhaled steroids within the last 4 weeks 8. Therapy with short- or long-acting inhaled betamimetic within the last 4 weeks 9. Therapy with leukotriene antagonists within the last 4 weeks 10. Therapy with inhaled anticholinergics within the last 4 weeks 11. girls of childbearing age (defined from Tanner stage ≥ B3)
Test product(s)	Tiotropium inhalation solution (5 µg) via Respimat® <u>Dose of administration:</u> 5 µg per day <u>Mode of administration:</u> oral inhalation

	Batch number(s): B211000532/007139, B231000893/206007 Batch number(s) Respimat® Inhaler A 5.7.1: B201002593/20L5024, B231001679/22L5037
Reference therapy	Placebo Dose of administration: not applicable Mode of administration: oral inhalation Batch number(s): B211000318/006140, B231000899/301718 Batch number(s) Respimat® Inhaler A 5.7.1: B201002593/20L5024, B231001679/22L5037
Duration of treatment	Treatment phase with tiotropium Product: Tiotropium inhalation solution (5 µg) via Respimat® Dose: Tiotropium daily 5 µg (2 doses of 2,5 µg each) Duration: 12 weeks Treatment phase with placebo Product: Placebo via Respimat® Dose: Placebo: not applicable Duration: 12 weeks Treatment arm **tiotropium-placebo** week 1 to 12 treatment phase with tiotropium week 13 to 14 wash out phase week 15 to 26 treatment phase with placebo Treatment arm **placebo-tiotropium** week 1 to 12 treatment phase with placebo week 13 to 14 wash out phase week 15 to 26 treatment phase with tiotropium

2 INDIVIDUAL STUDY TABLE

Not applicable.

3 INVESTIGATORS AND TRIAL SITES

No. of Trial Site	Trial Site	Investigator(s)
01	Medizinische Fakultät der TU Dresden, Klinik und Poliklinik für Kinder- und Jugendmedizin, Fetscherstr. 74, 01307 Dresden	Prof. Dr. med. Christian Vogelberg
02	Universitätsklinikum Augsburg, Klinik für Kinder- und Jugendmedizin, Stenglinstr. 2, 86156 Augsburg	Dr. med. Michael Gerstlauer

4 METHODOLOGY

The TRIBOR study was a randomized, double-blind, placebo-controlled phase II study with a cross-over design and was planned as a multicentre trial.

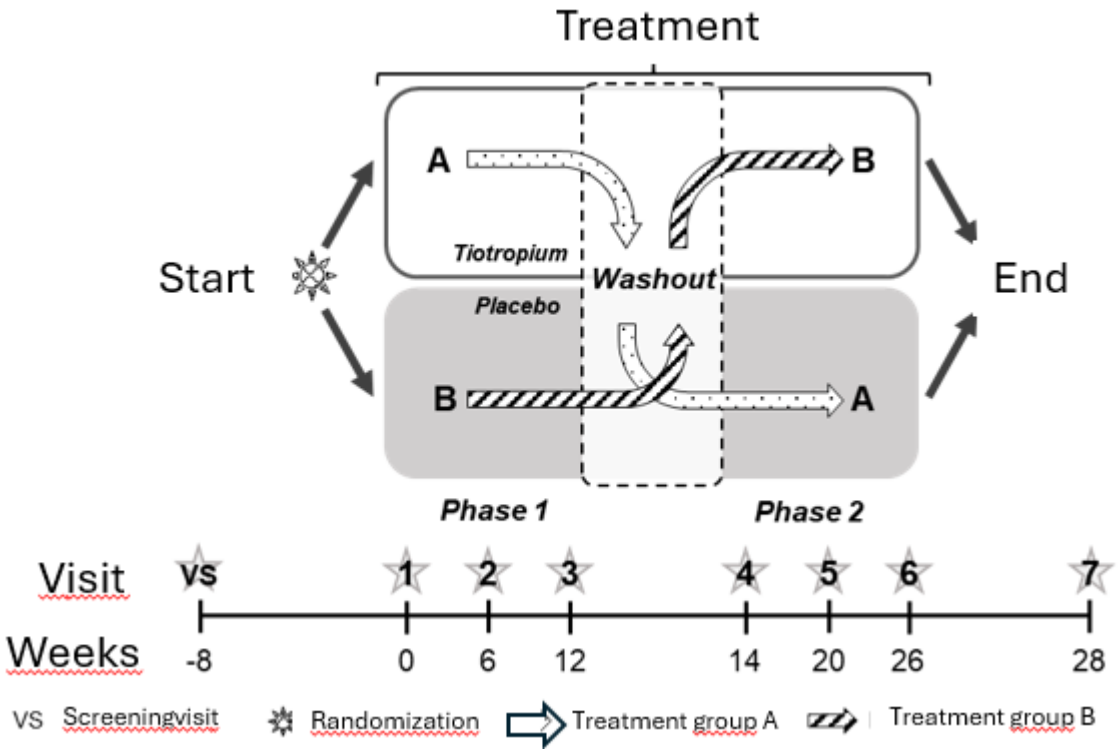


Figure 1 Study design

At the beginning, any previous treatment and the inclusion and exclusion criteria were checked and the screening visit was carried out. Randomization into one of two treatment groups took place at the baseline visit:

- the tiotropium-placebo group initially received the investigational product for 12 weeks (daily dose of 5µg tiotropium). After a two-week wash-out phase, these patients received the placebo for 12 weeks.
- In the placebo-tiotropium group, the order of investigational product and placebo was swapped (cross-over design).

Randomization was performed by block randomization with variable block length and was stratified by trial site. The randomization list was generated by KKS Dresden using nQuery Advisor®.

Patients, investigators, medical personnel and all persons involved in the evaluation of the study were blinded to allocated study therapy until database lock. Boehringer Ingelheim Pharma GmbH&Co. KG manufactured medication in a blinded manner and delivered it to the trial centres. Sealed envelopes for unblinding were provided to trial centres and pharmacovigilance department of KKS Dresden by Boehringer Ingelheim Pharma GmbH&Co KG.

Since many of the children involved in the study had behavioral abnormalities due to preterm birth, correct inhalation manoeuvre and inhalation technique were monitored regularly.

Lung function parameters were measured at the beginning, in the middle and at the end of each treatment phase. PEF was measured daily at home in the morning and in the evening.

The MasterScope CT spirometer and treadmill were used to measure lung function and perform treadmill provocation. The criteria for the use, performance, and daily calibration of the spirometer were based on the criteria of the American Thoracic Society and European Respiratory Society (ATS/ERS). Spirometry was performed in a sitting position. For optimal measurement of FEV_1 and FVC, serial measurements (a maximum of five measurements) were performed to exclude artefacts (e.g. cough artefact), and the best 3 manoeuvres with the highest FEV_1 and FVC values were used for the measurement. The MEF_{75-25} values depended on the validation of the FVC value; therefore, the highest measured FEV_1 and FVC values were used for the calculation.

At the beginning of treatment phase 1 and at the end of treatment phase 1 and 2, the lung function test was extended to include treadmill provocation. Patients received a lung function measurement as described above, after which they had to walk on a treadmill and exert themselves according to the standardized procedure based on international ATS/ERS criteria. After 5 and 10 minutes, lung function was measured again in order to record the differences in FEV_1, FVC, MEF_{75-25}, and other lung function parameters.

The parameters measured to assess lung function are common and generally accepted by international scientific community.
Fourteen days after the end of treatment phase 2, a final examination (V7) was carried out.

Onsite and central monitoring were carried out as part of the clinical trial for quality control purposes.

5 STATISTICAL METHODS

The final analysis was planned in detail in the statistical analysis plan (version 1.0F as of 28th January 2025, version 2.0F as of 24th February 2025). Here, only the most important specifications are presented.

Originally, the study was planned with a significance level of 5 percent and a power of 80 percent to detect an increase of the change of FEV_1 peak (0-1h) by 100 mL with an assumed standard deviation of 270 mL. A sample size of 60 evaluable patients was required. The standard deviation of the primary endpoint was reviewed by an independent external biometrician leading to a reduction of the assumed standard deviation to 150 mL. In consequence, the sample size calculation was revised in March 2023. Now, a sample size of 10 evaluable patients per randomization group (20 evaluable patients in total) were needed to reach the power of 80 percent in the confirmatory analysis. Assuming a drop out rate of 20 percent, 26 patients were planned to be recruited.

An interim analysis of efficacy was neither planned nor conducted. In the confirmatory analysis, one statistical test was performed in one analysis population, namely FAS population. Thus, no type-one-error adjustment was required.

Study Populations:

- Full analysis set (FAS) consists of all randomized patients who started at least one treatment sequence.
- Per-protocol set (PPS) consists of all patients of the FAS population without any severe protocol deviation
    - violation of inclusion or exclusion criteria apart from exclusion criteria 7 to 10 if administered for a short term only
    - inhalation of tiotropium instead of placebo in the placebo treatment phase
    - inhalation of placebo instead of tiotropium in the tiotropium treatment phase
    - inadequate adherence to study therapy prior to lung function test, i.e. no inhalation for more than 2 consecutive days directly before at the end of treatment phase visit (V3 or V6, respectively)
    - study visit at end of treatment phase (namely V3 and V6) carried out too early, i.e. less than 3 weeks after start of corresponding therapy phase
    - forbidden concomitant medication
- Safety analysis set consists of all subjects who started at least one treatment phase. Thus, in this trial, safety analysis set and FAS population are identical.

Confirmatory analysis of primary endpoint (FAS):

Calculation: The primary outcome of efficacy was the maximally forced expiratory volume in one second FEV_1 (one second capacity). This lung function parameter was measured after inhalation with the Respimat® after 30 and 60 minutes (before any standardized treadmill provocation). The maximum of these two measurements is called **FEV_1 peak (0-1h)**.

For the primary endpoint, FEV_1 peak (0-1h) was measured at visits V1 and V4, i.e. at the beginning of the 12-week intervention phase, and after the end of the therapy phase with tiotropium and after administration of placebo (visit V3 and V6, respectively).

The differences in FEV_1 peak (0-1h) between visit V1 and V3 (treatment phase 1) and between visit V4 and V6 (treatment phase 2) provide the primary endpoint of the clinical trial.

Based on these values and depending on the randomization group, the difference between verum and placebo phase was calculated.

Missing values of the difference between verum and placebo phase were imputed in the confirmatory analysis with the fully conditional specification (FCS) regression method (Brand 1999; van Buuren 2007) taking into account age, gender and the randomization group (SAS procedure PROC MI, FCS REG).

Number of imputed data sets corresponds approximately to the relative frequency of missing values, i.e. if the primary endpoint is missing in about 5 percent of FAS patients, then 5 imputed data sets are generated (NIMPUTE option).

Analysis: First, the primary endpoint was described and presented graphically for both intervention phases (without imputed values). Primary endpoint data was checked for skewness and variance inhomogeneity. The t-test for paired data was then carried out for each imputed data set, taking into account the period effect (randomisation group), without further adjustment. (A carry-over effect was not investigated as the wash-out phase of 2 weeks was 7-fold longer than reported half-life of the drug (27 - 45 hours) (Senn 1992)). Finally, results of all imputed data sets were aggregated and the confirmatory test was performed.

Sensitivity analyses of primary endpoint:

- FCS regression method accounting for age, and sex but not randomization group
- FCS regression method considering age, sex, randomization group and absolute change of FEV1 after inhalation of salbutamol in the screening visit
- Complete case analysis
- per protocol analysis

Secondary endpoints of efficacy:

In the same way as the primary endpoint, the following secondary efficacy parameters were investigated:

- change in FEV_1 measured before inhalation after 12 weeks of treatment
- change in FEV_1 AUC (0-1h) after 12 weeks of treatment
- change in FVC peak (0-1h) after 12 weeks of treatment
- change in FVC measured before inhalation after 12 weeks of treatment
- change in FVC AUC(0-1h) after 12 weeks of treatment
- change in FEV_1/FVC (Tiffeneau-Pinelli index) measured before inhalation after 12 weeks of treatment
- change in MEF_{75-25} measured before inhalation after 12 weeks of treatment
- difference of FEV_1 measurement before and 5 minutes after standardized treadmill provocation after 12 weeks of treatment (no treadmill provocation at start of second phase planned, therefore, only values at the end of each treatment phase available)
- difference of FEV_1 measurement before and 10 minutes after standardized treadmill provocation after 12 weeks of treatment (no treadmill provocation at start of second phase planned, therefore, only values at the end of each treatment phase available)
- difference of FVC measurement before and 5 minutes after standardized treadmill provocation after 12 weeks of treatment (no treadmill provocation at start of second phase planned, therefore, only values at the end of each treatment phase available)
- difference of FVC measurement before and 10 minutes after standardized treadmill provocation after 12 weeks of treatment (no treadmill provocation at start of second phase planned, therefore, only values at the end of each treatment phase available)

- difference of MEF_{75-25} measurement before and 5 minutes after standardized treadmill provocation after 12 weeks of treatment (no treadmill provocation at start of second phase planned, therefore, only values at the end of each treatment phase available)
- difference of MEF_{75-25} measurement before and 10 minutes after standardized treadmill provocation after 12 weeks of treatment (no treadmill provocation at start of second phase planned, therefore, only values at the end of each treatment phase available)
- change in PEF measured in the morning at home after 12 weeks of treatment
- change in PEF measured in the evening at home after 12 weeks of treatment
- change in PEF variability (based on measurements at home in the morning and evening) after 12 weeks of treatment

Secondary endpoint PAQ-C: (patient reported outcome (Crocker et al. 1997, Kowalski KC 2004))

Originally, it was planned to analyse the questionnaire just like all secondary endpoints but without imputation. Finally, only differences of -1, 0 and 1 point(s) were observed between treatment phases. Therefore, the questionnaire was analysed by methods of categorical data (absolute and relative frequencies and McNemar test).

(Serious) adverse events:

Description of absolute and relative frequencies of events and patients with an event by treatment phase. Adverse events were coded by MedDRA version 26.0.

Centre effects: not applicable. Two trial sites were initiated and screened patients. But only one trial site enrolled, randomized and treated patients.

Interim analyses:

Interim analyses of efficacy were neither planned nor carried out.

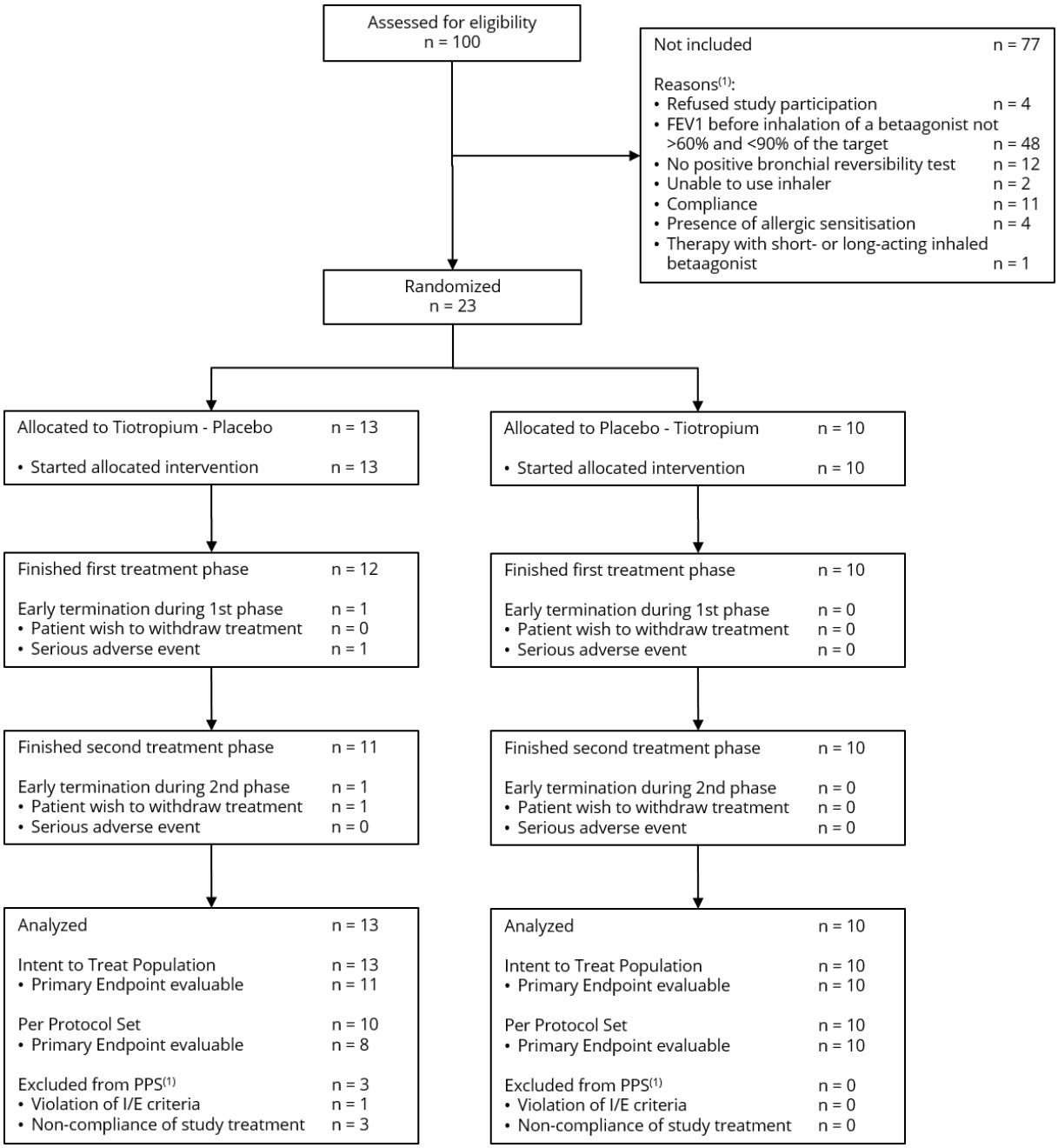
Safety was monitored by reporting of SAEs and annual safety reports to competent authorities.

6 RESULTS

Patients were recruited from July 7th, 2021 to December 13th, 2023. 100 patients were screened in 2 trial sites. Among these, 23 patients were eligible and randomized in this study. Finally, all 23 patients were randomized in only one trial site.

Originally, a sample size of 60 evaluable patients was planned (68 patients including 10 percent drop out rate). Given the slow recruitment rate the assumption regarding the standard deviation for the primary endpoint was reviewed by an external biometrician in March 2023. As a consequence the sample size calculation was revised and the study protocol was amended. With the sample size of 10 evaluable patients per randomization group (20 evaluable patients in total) a power of 80 percent was reached for the confirmatory analysis. Assuming a drop out rate of 20 percent, 26 patients were planned to be recruited.

6.1 CONSORT FLOW DIAGRAM



(1) Multiple reasons possible

Figure 2 CONSORT Flow Diagram

6.2 ANALYSIS POPULATIONS

Full analysis set comprises 23 patients. All of them were analysed for primary and secondary endpoints. Three patients with severe protocol deviations were excluded from per protocol set (for reasons see consort flow diagram in Figure 2). Hence, PPS comprises 20 patients.

6.3 BASELINE CHARACTERISTICS

23 patients were randomized with a median age of 9 years (minimum 6 years, maximum 12 years; 12/23 children 8 years or older). Fourteen male (61%) and nine female (39%) children were included.

In the following table, data are presented as absolute and relative frequency, or median and interquartile range.

Baseline Charactristics		FAS (N = 23)
Age at inclusion (yr)		9 [6 - 11]
Sex	male	14 (60.9%)
	female	9 (39.1%)
Height (m)		1.25 [1.16 - 1.43]
Weight (kg)		22.0 [19.5 - 32.3]
BMI (points)		14 [13 - 16]
Tanner Stage (only female patients)	< B3	9 (100.0%)
	>= B3	0 (0.0%)
Lung function at baseline		
FEV_1 before inhalation [L]		1.15 [1.05 - 1.59]
FEV_1 Peak (0-1h) [L]		1.21 [1.04 - 1.64]
FEV_1 AUC (0-1h) [mL]		1178 [1035 - 1620]
FVC before inhalation [L]		1.53 [1.33 - 1.97]
FVC Peak (0-1h) [L]		1.57 [1.35 - 2.03]
FVC AUC (0-1h) [mL]		1533 [1333 - 1945]
Tiffeneau-Pinelli index [%]		74 [69 - 81]
MEF_{75-25} before inhalation [L/s]		0.97 [0.72 - 1.19]
PAQ-C score at baseline		
filled out		23 (100%)
completely filled out		18 (78.3%)
	missing	5 (21.7%)
	1	0 (0.0%)
	2	9 (39.1%)
	3	8 (34.8%)
	4	1 (4.3%)
	5	0 (0.0%)

Table 1 Baseline characteristics

6.4 STUDY TREATMENT AND COMPLIANCE

All 23 patients started at least one treatment sequence.

Tiotropium phase (n=23): The median duration of verum treatment was 85 days (IQR: 81 to 90 days, range: 11 to 105 days). The median total dose of tiotropium was 425 µg (IQR: 400 to 450 µg; range 55 to 525 µg). One patient of randomization group “tiotropium - placebo” stopped study treatment during tiotropium phase due to an SAE (see consort flow diagram in chapter 6.1).

Placebo phase (n=22): The median duration of placebo phase was 83 days (IQR: 79 to 89 days, range: 1 to 97 days). The median “total dose” of placebo was 413 µg (IQR: 395 to 445

µg; range 5 to 485 µg). One patient of randomization group “tiotropium - placebo” discontinued study treatment during placebo phase at the parents’ request (see consort flow diagram in chapter 6.1).

Insufficient compliance with study treatment (definition see chapter 5) occurred in three patients. In all three cases, placebo phase was concerned.

None of the patients took forbidden concomitant medication.

6.5 PRIMARY ENDPOINT CHANGE IN FEV_1 PEAK (0-1H) AFTER 12 WEEKS OF TREATMENT

For each patient, first, the change in FEV_1 peak (0-1h) after treatment phase with tiotropium and after treatment phase with placebo were calculated. Afterwards, the difference of these values between tiotropium and placebo phase was calculated. A positive value indicates a higher change after tiotropium and a negative value indicates a lower change after tiotropium compared to placebo.

6.5.1 CONFIRMATORY ANALYSIS OF PRIMARY ENDPOINT

Two patients in the full analysis set prematurely terminated the clinical trial. In these cases, the data on primary endpoint were not completely documented. As prespecified in the study protocol and statistical analysis plan, multiple imputation of the primary endpoint was applied in the confirmatory analysis. According to SAP, ten imputations were calculated given a missing rate of about 10 percent (2/23 = 8.7%).

After multiple imputation, a treatment effect of 66.9 mL with a 95 percent confidence interval -3.4 mL to 137.3 mL was estimated, p-value = 0.062. Thus, tiotropium effect on primary endpoint was not statistically significant.

6.5.2 SENSITIVITY ANALYSES OF PRIMARY ENDPOINT

Furthermore, a complete case analysis in the full analysis set (n=21) was performed.

Treatment phase	**FEV_1 peak (0-1h)**		
	at the beginning [L]	**at the end [L]**	**change [mL]**
tiotropium (n=21)	1.41 ± 0.43	1.53 ± 0.44	122 ± 85
placebo (n=21)	1.27 ± 0.37	1.32 ± 0.41	52 ± 105

Table 2 FEV_1 peak (0-1h) complete case analysis in FAS

The following figure shows the primary endpoint for both phases by patient (complete cases).

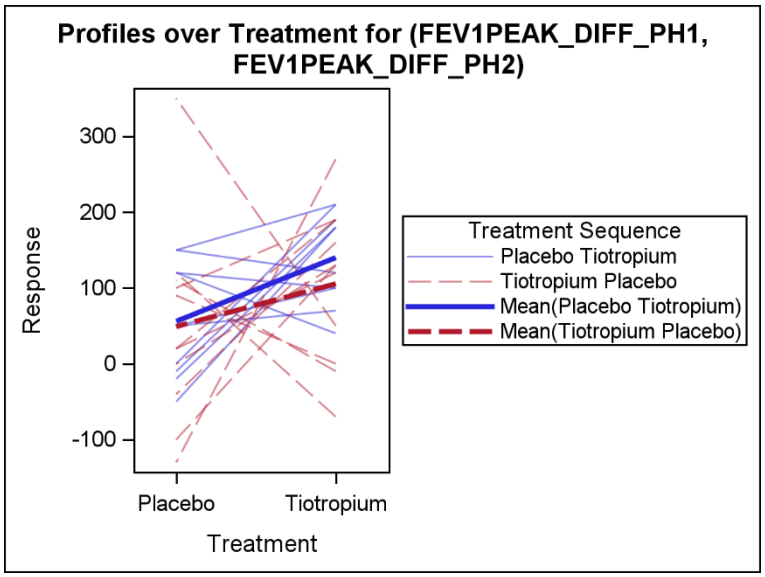


Figure 3 Change in FEV_1 peak (0-1h) after treatment sequences (FAS, complete case analysis)

Treatment effect in complete case analysis is reported in the next table along with further results of preplanned sensitivity analyses.

Sensitivity analysis of primary endpoint	Treatment Effect and 95% CI		p-value
FAS complete case analysis	70.2 mL	[-7.7; 148.1]	0.075
FAS multiple imputation accounting for age and sex	68.9 mL	[-0.02; 137.8]	0.050
FAS multiple imputation accounting for age, sex, randomization group, and absolute change of FEV1 after salbutamol at screening	68.8 mL	[-2.3; 139.9]	0.058
PPS multiple imputation accounting for age, sex and randomization group	64.4mL	[-12.5; 141.3]	0.101

Table 3 Primary endpoint of efficacy, sensitivity analyses

6.6 SECONDARY ENDPOINTS OF EFFICACY

6.6.1 SECONDARY ENDPOINTS OF EFFICACY, LUNG FUNCTION

In the following table, the treatment effect of tiotropium compared to placebo is reported. Thus, for each secondary endpoint, the treatment effect is the difference between tiotropium phase and placebo phase.

Secondary endpoint and kind of analysis	Treatment Effect and 95% CI		p-value
FEV_1 before inhalation [mL]			
FAS multiple imputation(1)	222.5	[130.3; 314.7]	<0.0001
FAS complete case	225.9	[125.1; 326.6]	0.0002
PPS multiple imputation(1)	227.4	[122.4; 332.4]	<0.0001
FEV_1 AUC (0-1h) [mL]			
FAS multiple imputation(1)	114.0	[40.2; 187.9]	0.003
FAS complete case	119.0	[37.9; 200.2]	0.006
PPS multiple imputation(1)	117.4	[34.8; 200.0]	0.005
FVC Peak (0-1h) [mL]			
FAS multiple imputation(1)	13.0	[-50.4; 76.4]	0.688
FAS complete case	14.3	[-56.9; 85.5]	0.680
PPS multiple imputation(1)	10.3	[-55.3; 75.9]	0.758
FVC before inhalation [mL]			
FAS multiple imputation(1)	87.3	[-16.0; 190.7]	0.098
FAS complete case	84.2	[-30.6; 199.0]	0.143
PPS multiple imputation(1)	85.6	[-30.1; 201.2]	0.147
FVC AUC (0-1h) [mL]			
FAS multiple imputation(1)	32.9	[-27.3; 93.0]	0.284
FAS complete case	32.5	[-35.3; 100.3]	0.328
PPS multiple imputation(1)	28.8	[-37.1; 94.6]	0.392
Tiffeneau-Pinelli index [%]			
FAS multiple imputation(1)	10.2	[5.9; 14.5]	<0.0001
FAS complete case	10.3	[5.5; 15.0]	0.0003
PPS multiple imputation(1)	10.7	[6.1; 15.4]	<0.0001

Secondary endpoint and kind of analysis	Treatment Effect and 95% CI		p-value
MEF_{75-25} before inhalation [mL/s]			
FAS multiple imputation(1)	436.7	[303.4; 570.0]	<0.0001
FAS complete case	444.6	[298.5; 590.7]	<0.0001
PPS multiple imputation(1)	448.9	[301.2; 596.6]	<0.0001
PEF in the morning(2) [L/min]			
FAS multiple imputation(1)	49.1	[18.0; 80.3]	0.002
FAS complete case	44.9	[10.4; 79.5]	0.014
PPS multiple imputation(1)	45.7	[14.7; 76.8]	0.004
PEF in the evening(2) [L/min]			
FAS multiple imputation(1)	14.1	[-10.5; 38.6]	0.262
FAS complete case	7.2	[-23.0; 37.4]	0.621
PPS multiple imputation(1)	19.4	[-5.7; 44.4]	0.129
PEF before inhalation(3) [L/min]			
FAS multiple imputation(1)	39.2	[22.8; 55.7]	<0.0001
FAS complete case	39.0	[20.8; 57.2]	0.0003
PPS multiple imputation(1)	39.3	[21.2; 57.5]	<0.0001
PEF variability [L/min]			
FAS multiple imputation(1)	-1.7	[-24.4; 21.1]	0.884
FAS complete case	-2.2	[-28.4; 24.0]	0.860
PPS multiple imputation(1)	-1.2	[-27.0; 24.5]	0.925
change of FEV_1 5 min after treadmill provocation [mL]			
FAS multiple imputation(1)	1.6	[-52.3; 55.5]	0.954
FAS complete case	6.3	[-52.5; 65.1]	0.824
PPS multiple imputation(1)	-2.1	[-62.9; 58.7]	0.945
change of FEV_1 10 min after treadmill provocation [mL]			
FAS multiple imputation(1)	-28.3	[-71.3; 14.6]	0.196
FAS complete case	-31.0	[-77.9; 15.9]	0.182
PPS multiple imputation(1)	-27.7	[-75.6; 20.2]	0.257

Secondary endpoint and kind of analysis	Treatment Effect and 95% CI		p-value
change of FVC 5 min after treadmill provocation [mL]			
FAS multiple imputation(1)	-6.0	[-84.3; 72.3]	0.881
FAS complete case	-1.2	[-84.8; 82.4]	0.977
PPS multiple imputation(1)	6.1	[-77.9; 90.1]	0.886
change of FVC 10 min after treadmill provocation [mL]			
FAS multiple imputation(1)	-15.1	[-84.4; 54.3]	0.670
FAS complete case	-6.0	[-77.8; 65.9]	0.864
PPS multiple imputation(1)	-1.4	[-74.4; 71.5]	0.969
change of MEF_{75-25} 5 min after treadmill provocation [mL/s]			
FAS multiple imputation(1)	-70.4	[-175.8; 35.1]	0.191
FAS complete case	-68.9	[-181.2; 43.4]	0.214
PPS multiple imputation(1)	-74.6	[-191.3; 42.1]	0.210
change of MEF_{75-25} 10 min after treadmill provocation [mL]			
FAS multiple imputation(1)	-166.0	[-234.8; -97.3]	<0.0001
FAS complete case	-165.3	[-241.7; -88.9]	0.0002
PPS multiple imputation(1)	-162.4	[-232.7; -92.2]	<0.0001

(1) multiple imputation accounting for age, sex and treatment sequence
(2) measured at home
(3) measured by lung function test

Table 4 Secondary endpoints of efficacy

In summary, a statistically significant improvement after treatment with tiotropium was observed for the secondary endpoints of efficacy FEV_1 before inhalation, FEV_1 AUC (0-1h), Tiffeneau-Pinelli index, MEF_{75-25} before inhalation, PEF measured in the morning at home, PEF measured during lung function test before inhalation, and change of MEF_{75-25} 10 min after treadmill provocation.

Results were consistent for all sensitivity analyses planned in the full analysis set as well as per protocol set, including sensitivity analyses not presented in this synopsis.

6.6.2 PHYSICAL ACTIVITY QUESTIONAIRE FOR CHILDREN

In the full analysis set, the physical activity questionnaire for children (PAQ-C) was completely documented after both treatment phases in 15/23 patients (65%) only, although basically filled out in 20/23 patients. The manual of PAQ-C does not contain any specifications for handling of missing data. Multiple imputation was not planned for this secondary endpoint.

In 9 patients, the PAQ-C score after tiotropium and after placebo were equal. In 2 patients, the score was 1 point lower after tiotropium and in 4 patients, the score was 1 point higher after tiotropium compared to placebo phase (exact Mc Nemar test in complete case analysis p=0.688).

		PAQ-C score at the end of placebo phase		
		2 points	**3 points**	**Number of patients**
PAQ-C score at the end of tiotropium phase	**2 points**	5	2	7
	3 points	4	4	8
	Number of patients	9	6	15

Table 5 PAQ-C score, complete case analysis in FAS

Per protocol set: the PAQ-C was completely documented after both treatment phases in 12/20 patients (60%). In 9 patients, the PAQ-C score after tiotropium and after placebo were equal. In 1 patient, the score was 1 point lower after tiotropium and in 2 patients, the score was 1 point higher after tiotropium compared to placebo phase (exact Mc Nemar test in complete case analysis p=1.000).

In contrast to the statistical analysis plan, the endpoint was not investigated by t-test for paired data. This is due to the fact that only scores of 2 and 3 points were observed at the end of treatment phases. Thus, the differences amount to -1, 0 and 1 point(s) and the endpoint was investigated by methods for categorical data.

6.7 SECONDARY ENDPOINTS OF SAFETY

In the TRIBOR study safety analysis set and full analysis set are identical. All 23 patients started treatment sequence with tiotropium and 22 patients with placebo.

All 23 patients of the full analysis set had at least one adverse event. In total, 157 events were reported.

During the tiotropium phase, 84 events were reported in 20 patients and during placebo phase, 73 events in 21 patients.

None of the adverse events was assessed as (possibly) related to investigational medicinal product by the investigator.

Two adverse events of grade 3 were reported. Both occurred during treatment with tiotropium and were serious adverse events (MedDRA preferred term of these events: intraocular pressure increased, febrile convulsion).

Study treatment was withdrawn in 2 cases due to (serious) adverse event (AE character change during placebo phase, SAE intraocular pressure increased during tiotropium phase).

No SAR, and hence SUSAR, was reported during the trial.

The following tables report the frequencies of adverse events and number of patients affected by treatment phase, tiotropium sequence first followed by placebo phase.

Adverse events during tiotropium phase

System Organ Class	Preferred Term	Events	Patients
Blood and lymphatic system disorders		2	2 / 23 (8.7%)
	Iron deficiency anaemia	1	1 / 23 (4.3%)
	Lymphadenopathy	1	1 / 23 (4.3%)
Cardiac disorders		1	1 / 23 (4.3%)
	Palpitations	1	1 / 23 (4.3%)
Ear and labyrinth disorders		1	1 / 23 (4.3%)
	Tympanic membrane hyperaemia	1	1 / 23 (4.3%)
Gastrointestinal disorders		15	9 / 23 (39.1%)
	Abdominal pain	2	2 / 23 (8.7%)
	Abdominal pain upper	3	3 / 23 (13.0%)
	Diarrhoea	3	3 / 23 (13.0%)
	Gastritis	2	2 / 23 (8.7%)
	Nausea	5	3 / 23 (13.0%)
General disorders and administration site conditions		4	4 / 23 (17.4%)
	Malaise	1	1 / 23 (4.3%)
	Pyrexia	3	3 / 23 (13.0%)
Infections and infestations		34	15 / 23 (65.2%)
	Gastrointestinal infection	1	1 / 23 (4.3%)

System Organ Class	Preferred Term	Events	Patients
	Influenza	1	1 / 23 (4.3%)
	Nasopharyngitis	12	10 / 23 (43.5%)
	Rhinitis	15	6 / 23 (26.1%)
	Upper respiratory tract infection	1	1 / 23 (4.3%)
	Respiratory tract infection	1	1 / 23 (4.3%)
	Tonsillitis bacterial	1	1 / 23 (4.3%)
	COVID-19	2	2 / 23 (8.7%)
Investigations		3	2 / 23 (8.7%)
	Body temperature increased	1	1 / 23 (4.3%)
	Intraocular pressure increased(1)	1	1 / 23 (4.3%)
	Blood pressure difference of extremities	1	1 / 23 (4.3%)
Musculoskeletal and connective tissue disorders		2	2 / 23 (8.7%)
	Back pain	1	1 / 23 (4.3%)
	Pain in extremity	1	1 / 23 (4.3%)
Nervous system disorders		9	6 / 23 (26.1%)
	Febrile convulsion(1)	1	1 / 23 (4.3%)
	Headache	7	4 / 23 (17.4%)
	Psychomotor hyperactivity	1	1 / 23 (4.3%)
Respiratory, thoracic and mediastinal disorders		13	11 / 23 (47.8%)
	Bronchial obstruction	1	1 / 23 (4.3%)
	Cough	7	6 / 23 (26.1%)
	Dyspnoea	1	1 / 23 (4.3%)
	Pharyngeal erythema	1	1 / 23 (4.3%)
	Oropharyngeal pain	3	3 / 23 (13.0%)

(1) Serious adverse event

Table 6 Phase tiotropium: Frequency of (patients with) adverse events

Adverse events during placebo phase

System Organ Class	Preferred Term	Events	Patients
Blood and lymphatic system disorders		3	3 / 22 (13.6%)
	Lymphadenopathy	3	3 / 22 (13.6%)
Gastrointestinal disorders		4	3 / 22 (13.6%)
	Abdominal pain	2	2 / 22 (9.1%)
	Gastrointestinal disorder	1	1 / 22 (4.5%)
	Vomiting	1	1 / 22 (4.5%)
General disorders and administration site conditions		2	2 / 22 (9.1%)
	Malaise	1	1 / 22 (4.5%)
	Pyrexia	1	1 / 22 (4.5%)
Infections and infestations		34	17 / 22 (77.3%)
	Conjunctivitis	2	2 / 22 (9.1%)
	Influenza	1	1 / 22 (4.5%)
	Nasopharyngitis	20	11 / 22 (50.0%)
	Rhinitis	8	5 / 22 (22.7%)
	Tonsillitis	1	1 / 22 (4.5%)
	Upper respiratory tract infection	1	1 / 22 (4.5%)
	COVID-19	1	1 / 22 (4.5%)
Injury, poisoning and procedural complications		1	1 / 22 (4.5%)
	Contusion	1	1 / 22 (4.5%)
Investigations		4	3 / 22 (13.6%)
	Body temperature increased	4	3 / 22 (13.6%)
Nervous system disorders		6	3 / 22 (13.6%)
	Headache	5	3 / 22 (13.6%)
	Muscle spasticity	1	1 / 22 (4.5%)
Psychiatric disorders		1	1 / 22 (4.5%)
	Personality change	1	1 / 22 (4.5%)
Respiratory, thoracic and mediastinal disorders		15	9 / 22 (40.9%)
	Cough	10	7 / 22 (31.8%)
	Rhinorrhoea	1	1 / 22 (4.5%)
	Oropharyngeal pain	2	2 / 22 (9.1%)
	Throat clearing	2	1 / 22 (4.5%)
Skin and subcutaneous tissue disorders		3	2 / 22 (9.1%)
	Dry skin	1	1 / 22 (4.5%)
	Pruritus	2	1 / 22 (4.5%)

Table 7 Phase placebo: Frequency of (patients with) adverse events

7 CONCLUSION

The aim of this study was to investigate the efficacy of tiotropium in terms of changes in lung function parameters and the safety profile of tiotropium in patients with bronchopulmonary dysplasia (BPD) when used as monotherapy. No statistically significant change was observed in the primary endpoint, which was the change in peak FEV_1(0-1h) (treatment effect of tiotropium 66.9 mL, 95% CI (-3.4 mL; 137.3 mL), p = 0.062). However, for secondary endpoints—including FEV_1(0), the area under the curve of FEV_1, Tiffeneau Pinelli index, PEF(0) measured during lung function, PEF measured in the morning at home, as well as MEF_{75-25}(0) values—a statistically significant improvement favouring tiotropium was observed. These changes are also clinically relevant, since they reflect aspects of bronchodilation in the lung function, and the patient might benefit from improved lung function in daily life, especially during physical exercise. Changes related to the FVC parameter were not statistically significant.

Although, frequent AEs occurred in both groups during the study, none of them was assessed as related to the investigational medicinal product by the investigator. In children of this age, concomitant infections (especially upper respiratory tract infections, fever and gastrointestinal infections) occur frequently. In patients with bronchopulmonary dysplasia, infections might present more severe due to the bronchial hyperreactivity. Both of the serious adverse events (SAEs) occurred within the tiotropium phase, however, the increased intraocular pressure might more likely be a consequence of preterm birth, while febrile seizures can occur in every otherwise healthy child. In our patient, the febrile seizure occurred in the context of a febrile respiratory infection. An increased risk associated with tiotropium use could not be identified.

A limiting factor in this study was the small number of patients, mainly due to a high rate of normal lung function or due to failure to respond to bronchodilation. Further problems in recruitment were due to the COVID-19 pandemia and lost contact to probably suitable patients.

In our study, the treatment effect in favour of tiotropium was marginally not significant in the primary endpoint. However, positive treatment effects of tiotropium on further relevant lung function parameters in children with BPD were observed in secondary endpoints. Taking all together, these results indicate a favourable impact of tiotropium which should not be ignored. Until today, to the best of our knowledge, there are no further studies who suggest this effect. Furthermore, there are currently no approved therapies for the treatment of BPD beyond neonatal and infant age.

This first study on tiotropium in BPD demonstrates that this treatment is safe and might lead to lung function improvement. Further studies are needed before a treatment recommendation with tiotropium for BPD can be made.

8 PUBLICATIONS

None.

9 SIGNATURES

The signing persons approve the report presented here by their signature. The described clinical trial was conducted according to the Declaration of Helsinki, Good Clinical Practice (GCP) as well as the applicable legal regulations.

Sponsor

Prof. Dr. med. Christian Vogelberg		
Name in block letters	Place, Date	Signature

Biostatistics

Evelyn Trips (Dipl.-math.)		
Name in block letters	Place, Date	Signature

10 LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse event of special interest
AMG	Arzneimittelgesetz
AR	Adverse reaction
ATS	American Thoracic Society
AUC	Area under the curve
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BMI	Body mass index
BPD	Bronchopulmonary dysplasia
CI	Confidence interval
COVID-19	Coronavirus disease 2019
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
eCRF	Electronic Case Report Form
ERS	European Respiratory Society
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FAS	Full analysis set
FCS	Fully conditional specification
FEV	Forced expiratory volume
FEV_1	Forced expiratory volume in one second
FPFV	First patient first visit
FVC	Forced vital capacity
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
I/E	Inclusion / exclusion
IMP	Investigational medicinal product
IQR	Interquartile range
ISF	Investigator site file
ITT	Intention to treat
KKS	Koordinierungszentrum für Klinische Studien
LPLV	Last patient last visit
MedDRA	Medical Dictionary for Regulatory Activities
MEF	Maximum expiratory flow
NA	Not applicable
ND	Not done
PAQ-C	Physical Activity Questionnaire for Children
PEI	Paul-Ehrlich-Institut
PEF	Peak Expiratory Flow

PPS	Per protocol set
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SAR	Serious Adverse Reaction
SAS	Statistical software SAS for Windows (“Statistical Analysis System”)
SDV	Source data verification
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UAR	Unexpected Adverse Reaction

11 REFERENCES

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