Summary Attachment

Clinical trial: EudraCT 2025-003399-58

Date: 14 August 2024

The above trial was ended prematurely, due to slow recruitment due to COVID-19. Recruitment ended March 2022, as decided by the steering committee. This decision was endorsed by the Data and Safety Monitoring Board.

A total of 49 patients were recruited and randomized. The last patient's last visit was in March 2023. Results from the 49 patients was published as a medical journal article (in Respiratory Research) in June 2024.

Thus, the result from the trial is reported as this "Summary Attachment", containing the brief summary above, and a copy of the published article below.

Eklöf *et al. Respiratory Research* (2024) 25:236
https://doi.org/10.1186/s12931-024-02860-9

Respiratory Research

RESEARCH Open Access

Systemic antibiotics for *Pseudomonas aeruginosa* infection in outpatients with non-hospitalised exacerbations of pre-existing lung diseases: a randomised clinical trial

Josefin Eklöf[1*], Imane Achir Alispahic[1], Karin Armbruster[1], Therese Sophie Lapperre[2,3,4], Andrea Browatzki[5], Rikke Holmen Overgaard[5], Zitta Barrella Harboe[5,11], Julie Janner[6], Mia Moberg[6], Charlotte Suppli Ulrik[6], Helle Frost Andreassen[2], Ulla Møller Weinreich[7], Jakob Lyngby Kjærgaard[1], Jenny Villadsen[1], Camilla Sund Fenlev[4], Torben Tranborg Jensen[8], Christina Wellendorph Christensen[9], Jette Bangsborg[9], Christian Ostergaard[10], Khaled Saoud Ali Ghathian[10], Alexander Jordan[1], Tobias Wirenfeldt Klausen[1], Thyge Lynghøj Nielsen[5], Torgny Wilcke[1], Niels Seersholm[1], Pradeesh Sivapalan[1,11] and Jens-Ulrik Stæhr Jensen[1,11]

Abstract

Background The effect of dual systemic antibiotic therapy against *Pseudomonas aeruginosa* in patients with pre-existing lung disease is unknown. To assess whether dual systemic antibiotics against *P. aeruginosa* in outpatients with COPD, non-cystic fibrosis (non-CF) bronchiectasis, or asthma can improve outcomes.

Methods Multicenter, randomised, open-label trial conducted at seven respiratory outpatient clinics in Denmark. Outpatients with COPD, non-CF bronchiectasis, or asthma with a current *P. aeruginosa*-positive lower respiratory tract culture (clinical routine samples obtained based on symptoms of exacerbation not requiring hospitalisation), regardless of prior *P. aeruginosa*-status, no current need for hospitalisation, and at least two moderate or one hospitalisation-requiring exacerbation within the last year were eligible. Patients were assigned 1:1 to 14 days of dual systemic anti-pseudomonal antibiotics or no antibiotic treatment. Primary outcome was time to prednisolone or antibiotic-requiring exacerbation or death from day 20 to day 365.

Results The trial was stopped prematurely based in lack of recruitment during the COVID-19 pandemic, this decision was endorsed by the Data and Safety Monitoring Board. Forty-nine outpatients were included in the study. There was a reduction in risk of the primary outcome in the antibiotic group compared to the control group (HR 0.51 (95%CI 0.27–0.96), $p = 0.037$). The incidence of admissions with exacerbation within one year was 1.1 (95%CI 0.6–1.7) in the dual antibiotic group vs. 2.9 (95%CI 1.3–4.5) in the control group, $p = 0.037$.

*Correspondence:
Josefin Eklöf
josefin.viktoria.ekloef@regionh.dk
Full list of author information is available at the end of the article

Conclusions Use of dual systemic antibiotics for 14 days against *P. aeruginosa* in outpatients with chronic lung diseases and no judged need for hospitalisation, improved clinical outcomes markedly. The main limitation was the premature closure of the trial.

Trial Registration ClinicalTrials.gov, NCT03262142, registration date 2017–08-25.

Summary of the article's main point

This is the first randomised controlled trial to report that dual systemic anti-pseudomonal antibiotic treatment seems to be a well-tolerated and effective treatment for reducing exacerbations in patient with chronic lung disease and a *Pseudomonas aeruginosa*-positive lower airway sample culture.

Introduction

*Pseudomonas aeruginosa*is a Gram-negative bacterium that is associated with a considerable burden of symptoms, hospitalisation and death in patients with chronic pulmonary diseases, including COPD, non-cystic fibrosis (CF) bronchiectasis, and asthma [1–3].

Currently, clinical practice relies on data from observational studies, suggesting clinical benefits of systemic antibiotic treatment in patients with pre-existing lung disease, including findings extrapolated from the treatment of *P. aeruginosa*infections in children with cystic fibrosis, where dual systemic anti-pseudomonal therapy has become a key treatment [4–8]. Based on these studies, international guidelines for patients with bronchiectasis recommend targeted antibiotic interventions against *P. aeruginosa*, ranging from monotherapy to combination therapy [7]. For COPD and asthma, no recommendations have been made, probably based on the lack of available clinical data.

Thus, current recommendations rely on low grade evidence, and there is a need for clinical trial data to clarify whether systemic antibiotics, including dual treatment, against *P. aeruginosa* in patients with chronic pulmonary diseases can improve clinical outcomes. We therefore conducted a randomised, good clinical practice (GCP) monitored, controlled trial to determine whether dual systemic antibiotics against *P. aeruginosa* in patients with COPD, non-CF bronchiectasis, or asthma, and no current indication for hospital admission, can reduce antibiotic or prednisolone requiring exacerbations and death. We hypothesised that antibiotic treatment would lower the risk of exacerbations and mortality in outpatients with COPD, non-CF bronchiectasis, or asthma and a *P. aeruginosa* positive lower respiratory tract sample.

Methods

Study design and participants

The study is a multicenter, randomised, GCP monitored, controlled, open-label trial conducted in outpatients with COPD, non-CF bronchiectasis, or asthma with a *P. aeruginosa*-culture positive lower respiratory tract sample. The study was carried out at seven respiratory outpatient clinics in Denmark between October 2017, and March 2023. Outpatients with a *P. aeruginosa*-positive lower respiratory tract sample (sputum, tracheal secretion, bronchial secretion or bronchial alveolar lavage) obtained within the previous 30 days, regardless of prior *P. aeruginosa*-status, and with a physician-judged no need for hospitalisation, were systematically screened and consecutively invited to participate if they fulfilled inclusion criteria and no exclusion criteria (Additional file 1: study protocol). The study was approved by the Ethics Committees (H-15010949), the Danish Medicines Agency (EudraCT 2015–003399-58) and the Danish Data Protection Agency (HGH-2017–036), and was monitored by a national GCP unit. The trial is registered at ClinicalTrials.gov (NCT03262142). No financial incentive was provided to the investigators or participants.

Outpatients were randomly assigned 1:1 to either systemic dual antibiotic treatment (antibiotic group) or no antibiotic treatment (control group) and stratified by study site and age (≤70 years vs. >70 years) (see Appendix for details regarding randomization sequence). The antibiotic intervention consisted of 14 days of combination therapy with piperacillin/tazobactam 4/0.5 g, administered intravenously four times daily, and oral ciprofloxacin 500 mg twice daily. Intravenous ceftazidime or meropenem was used if piperacillin/tazobactam could not be used because of allergy or antibiotic resistance.

Procedures

Outpatients were screened based on results from routine microbiological examinations of the lower respiratory tract samples obtained from patients attending the outpatient clinics of the participating respiratory departments. Samples were ordered by clinical staff based on clinical symptoms of exacerbation of the underlying lung disease. Fever, fatigue, peripheral oxygen saturation, and tachypnoea at rest were used as parameters to guide the

staff when assessing the need for hospitalisation. Antibiotics were administered in-hospital, since home-treatment with intravenous antibiotics was not available for all study sites at the time of implementation. However, between 2020 and 2022, one site (initiated in 2017), was able to provide treatment at home. We allowed a delay of initiating antibiotic treatment for up to six days in initiating therapy, since there, per the eligibility criteria, was no clinical indication for admission. Baseline measurements were obtained on the calendar date of recruitment (day 1) and follow-up visits were scheduled on day 14, 30, 60, 90, and 365. COPD assessment test (CAT), body mass index (BMI), Medical Research Council (MRC) dyspnoea score, and spirometry were assessed at all visits. Blood samples were drawn at day 1 (baseline) and day 14, and the outpatients underwent a high-resolution CT of the lungs at day 14 assess radiological signs of bronchiectasis at baseline.

Outcomes

The primary outcome was *time to prednisolone and/or antibiotic requiring exacerbation, in a primary or secondary health care sector, or death from day 20 to day 365 from randomisation.* Death was incorporated in the primary outcome to avoid lead-time bias as the death rate is expected to be high in this population of outpatients with severe pulmonary disease and could thus be incorrectly interpreted as protective of exacerbation. We chose to register events after 20 days from randomisation to avoid misclassifying the study intervention as a fulfilment of the primary outcome. A co-primary outcome of "days alive and out of hospital within 365 days" was degraded to the first secondary outcome by the trial statistician in agreement with the trial leadership (JE and JUSJ) since this outcome would be severely underpowered because of the premature closure of the trial (see " Statistical analysis"). This was done before the database was unblinded to the analysis (see Additional file 1).

The secondary outcomes were: *1) days alive and without hospitalisation from day 20 to day 365 from randomisation, 2) death within 365 days from randomisation, 3) number of admissions with exacerbation within 365 days from randomisation (defined as referral to emergency room or hospitalisation* [9]*), 4) number of days with non-invasive ventilation or invasive ventilation within 90 days from randomisation, 5) microbiological cure at day 90 (defined as P. aeruginosa-negative sputum culture until day 90; no microbiological cure was defined as a P. aeruginosa-positive sputum culture before or at day 90), 6) clinical cure at day 14 (defined as improvement of clinical signs and symptoms related to P. aeruginosa before or on day 14; clinical failure was defined as persistent or worsening of clinical signs and symptoms related to P. aeruginosa before or on day 14), 7) change in CAT score from randomisation to day 90, 8) change in BMI from randomisation to day 90, 9) change in forced expiratory volume in the first second (FEV_1) from randomisation to day 90, and 10) decrease of ≥ 200 ml in FEV_1 from randomisation to day 365.*

Statistical analysis

Sample size

The sample size was calculated using a group-sequential design, allowing for one interim analysis at half target recruitment, with a power of 80% to avoid type II error at a two-sided 5% significance level. Based on estimates and indicative figures in previous literature, a total of 150 patients (75 patients in each group) were required for the trial (see Additional file 1 for details) [3, 10–12].

Analyses

Data were analysed using intention-to-treat (ITT) principles, including all available data, regardless of whether the participant received the intervention. The primary outcome was also analysed using a modified ITT analysis (in study participants who started but did not complete the intervention) and per protocol analysis (in study participants who completed the entire intervention). Completion of the intervention was defined as 14 days of antibiotic treatment in the dual systemic anti-pseudomonal antibiotic study group, and as no anti-pseudomonal treatment within 14 days from randomisation in the control group. Partial completion to intervention was defined as 1–13 days of antibiotic treatment in the antibiotic study group and ≥ 1 day of *P. aeruginosa*-active antibiotic treatment within 14 days from randomisation in the control group.

Data for the primary outcome analyses was analysed using an unadjusted Cox proportional hazard model and results reported as hazard ratio [HR] and 95% confidence limits. A Kaplan–Meier plot was used to describe the process of exacerbations and death in the study groups. A multivariable Cox proportional hazards model adjusting for sex (male vs. female), CAT-score (< 21 vs. ≥ 21) and FEV_1% predicted (< 50% vs. ≥ 50%) at randomisation was also conducted. Secondary outcomes were compared between the study groups using t-test or Mann–Whitney U test for continuous data and χ^2-test for nominal data. Analysis of covariance (ANCOVA) was used to model the effect of the intervention on changes in the mean of continuous outcomes, adjusting for the baseline value. All analyses were done using the statistical software SAS (version 9.4) and R (version 3.4.3). Sample size calculation was done using StudySize 2.0 (Frölunda, Sweden).

The interim analysis was planned at half target recruitment (75 patients), with a focus on reporting data on the primary outcome, all-cause mortality at day 365, microbiological cure at day 14 and assessment of study's

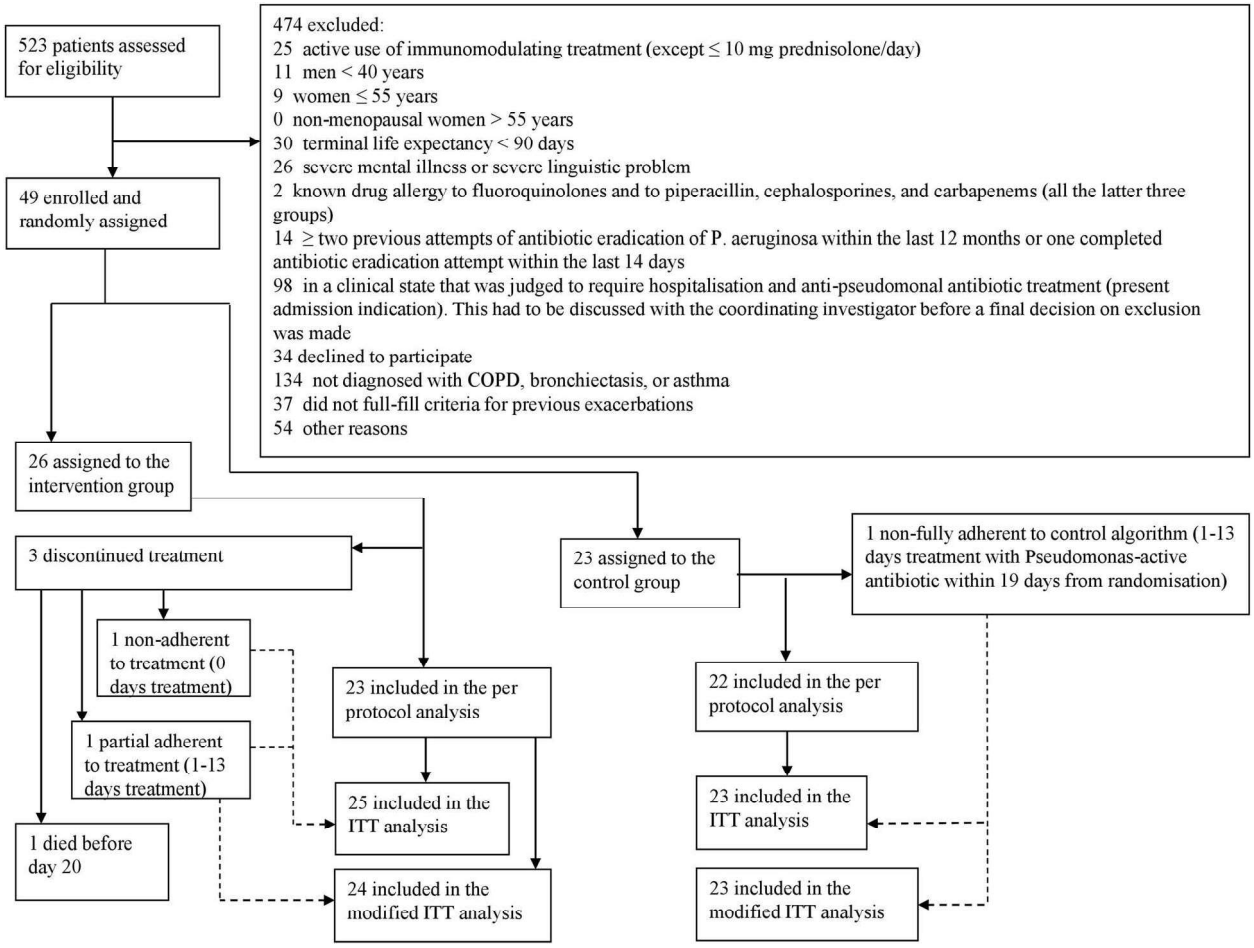


Fig. 1 CONSORT flow diagram

ITT intention-to-treat

futility. An independent data and safety monitoring board (DSMB) was appointed to review the trial's safety, efficacy, and progression (see Additional file 1). Due to the slow recruitment rate during the COVID-19 pandemic, the trial's steering committee decided to stop further recruitment in February 2022, when approximately 1/3 of the planned outpatients had been enrolled in the study. This decision was tested with the DSMB, who endorsed it (see Additional file 1). Due to the considerable reduction of the study size, the primary outcome was conducted solely as a "time to event" analysis, and the "days alive and out of hospital" analysis was degraded from a co-primary outcome to the first secondary outcome. As previously mentioned, this decision was made before data was unblinded to the analysis (see " Outcomes"). Data analyses were performed by an analysis team (TWK and AJ), including a trial statistician (TWK), after the final data from the last outpatients last follow-up visit was entered and the database was locked. All analyses were done before breaking of the randomisation code. The study group was presented to the results and unblinded at a scheduled unblinding-meeting after the analyses were performed.

Results

A total of 523 outpatients were screened between October 14, 2017, and February 23, 2022. Of these, 49 outpatients (9%) were recruited and randomly assigned to either the antibiotic group ($n=26$) or the control group ($n=23$) (Fig. 1). There was complete adherence to the intervention in 92% of the outpatients in the antibiotic group and 96% in the control group. All outpatients in the antibiotic group were treated with combination therapy, except for one case where intravenous monotherapy with meropenem was administered due to antibiotic resistance against ciprofloxacin. Baseline characteristics

Table 1 Baseline characteristics of the intention-to-treat population

	Antibiotic group ($n=26$)	Control group ($n=23$)	Number missing	*P*-value
Age (years), mean (SD)	72 (9)	71 (9)	0	0.80
Sex			0	1.00
Female, n (%)	12 (46)	11 (48)		
Male, n (%)	14 (54)	12 (52)		
Body mass index (kg/m2), median (IQR)	24 (21—28)	26 (21—34)	1	0.30
Smoking			0	0.48
Current, n (%)	1 (4)	2 (9)		
Past, n (%)	20 (77)	19 (83)		
Never, n (%)	5 (19)	2 (9)		
Pack years, median (IQR)	34 (30–40)	42 (30–50)	3	0.14
COPD, n (%)	20 (77)	21 (91)	0	0.25
Bronchiectasis, n (%)	12 (46)	8 (35)	0	0.56
Asthma, n (%)	8 (31)	3 (13)	0	0.18
Exacerbations 12 months prior to inclusion, total, median (IQR)	2 (2—2)	3 (2—5)	0	0.80
Pulmonary function and symptoms				
CAT score, mean (SD)	21 (7)	23 (6)	1	0.36
MRC, median (IQR)	3 (2—3)	3 (1—4)	1	0.56
FEV1 (L), median (IQR)	0.94 (0.67—1.25)	0.83 (0.67—1.19)	0	1.00
FEV1 (% predicted), median (IQR)	40 (30—51)	39 (28—52)	0	0.87
FVC (L), median (IQR)	2.07 (1.64—2.69)	1.93 (1.44—2.31)	0	0.61
FVC (% predicted), median (IQR)	69 (64—81)	66 (53—81)	0	0.67
FEV1/FVC ratio (%), median (IQR)	44 (37—58)	51 (36—58)	0	0.54
Home oxygen therapy, n (%)	2 (8)	5 (22)	0	0.23
Home NIV, n (%)	1 (4)	2 (9)	0	0.59
Increased dyspnea, n (%)	10 (38)	16 (70)	0	0.045
Increased cough, n (%)	13 (50)	13 (57)	0	0.78
Increased sputum volume, n (%)	9 (35)	13 (57)	0	0.16
Increased sputum purulence, n (%)	10 (38)	9 (39)	0	1.00
Comorbidities				
Ischemic heart disease, n (%)	1 (4)	0 (0)	0	1.00
Heart failure, n (%)	3 (12)	3 (13)	0	1.00
Diabetes, n (%)	0 (0)	6 (26)	0	0.0072
Chronic renal failure, n (%)	1 (4)	0 (0)	0	1.00
Primary immunodeficiency, n (%)	0 (0)	0 (0)	0	1.00
Activities of daily living			0	0.67
Score 0–1, n (%)	22 (85)	21 (91)		
Score 2–4, n (%)	4 (15)	2 (9)		
Current respiratory medication				
Long-acting β2 agonist	24 (92)	21 (91)	0	1.00
Long-acting muscarin antagonist	24 (92)	19 (83)	0	0.40
Inhaled corticosteroid	18 (69)	13 (57)	0	0.39
Maintenance oral corticosteroid ≤ 5 mg/day	3 (12)	4 (17)	0	0.69
Maintenance azithromycin	2 (8)	0 (0)	0	0.49
Maintenance inhaled antibiotics	0 (0)	1 (4)	0	0.47
Short-term antibiotics at time of enrolment *	3 (12)	6 (26)	0	0.27
Clinical findings				
Systolic blood pressure (mmHg), mean (SD)	141 (23)	139 (16)	1	0.91
Diastolic blood pressure (mmHg), mean (SD)	78 (12)	78 (11)	1	0.57
Heart rate (beats per minute), mean (SD)	81 (12)	90 (13)	1	0.029

Table 1 (continued)

	Antibiotic group ($n=26$)	Control group ($n=23$)	Number missing	*P*-value
Oxygen saturation (%), median (IQR)	94 (92—97)	95 (92—95)	2	0.35
Respiratory rate (breaths per minute), median (IQR)	18 (15—20)	19 (16—20)	1	0.23
Temperature (°C), median (IQR)	36.6 (36.1—37.0)	36.5 (35.8—37.1)	1	0.60

Data are n (%), median (IQR), or mean (SD) unless otherwise specified

COPD Chronic obstructive pulmonary disease, *CAT* COPD assessment test, *MRC* Medical research council dyspnea scale, *FEV*, Forced expiratory volume the first second, *FVC* Forced expiratory volume, *NIV* Non-invasive ventilation

* Antibiotics not active against *Pseudomonas aeruginosa*

in the two groups were overall well-balanced regarding demographics, pulmonary function and clinical findings (Table 1). The majority of outpatients had COPD, followed by non-CF bronchiectasis. All but one patient with asthma had a concurrent diagnosis of COPD, or bronchiectasis. A total of 15 (31%) patients were *P. aeruginosa*-naïve prior to the study. All outpatients except one (patient in the antibiotic group who died before day 20) entered the intention-to-treat analysis. There was a 100% follow-up on the primary outcome.

Primary outcome

Time to prednisolone or antibiotic requiring exacerbation or death from day 20 to day 365 from randomisation was increased in the antibiotic group compared to the control group in the intention-to-treat analysis ((HR) 0.51 (95% CI 0.27–0.96), $p=0.037$), Table 2). Figure 2 illustrates the survival probability using a Kaplan–Meier plot. The result remained stable in the multivariable adjusted Cox proportional hazard regression model, adjusting for sex, CAT-score (<21 vs. ≥21) and FEV1% predicted (<50% vs. ≥50%) at randomisation ((HR) 0.49 (95% CI 0.25–0.95), $p=0.034$). The signal was unchanged in the modified intention-to-treat analysis but did not reach statistical significance in the per-protocol analysis (Table 2). Due to the small sample size, the lack of statistical significance in the per-protocol analysis might reflect a lack of statistical power, and not a lack of treatment effect of the antibiotic invention. Prior *P. aeruginosa*-status (naïve versus non-naïve) did not alter the signal in the antibiotic group compared to the control group (incident rate ratio: 0.47 in naïve patients versus 0.13 in non-naïve patients). The results remained statistically significant in the population with COPD in a post-hoc analysis differentiating patients by the type of lung disease (Table 1 in Additional file 1).

Secondary outcomes

Numerically, the *days alive and without hospitalisation from day 20 to day 365* was higher in the dual systemic

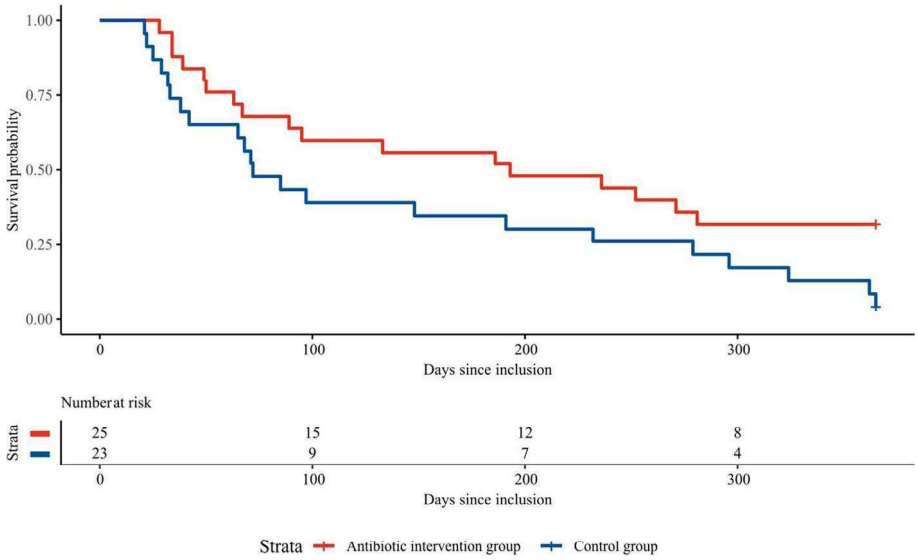


Fig. 2 Exacerbation and death from day 20 to 365

Table 2 Primary outcome measurement

	Antibiotic group ($n=26$)	Control group ($n=23$)			
Exacerbation or death within days 20 to 365	**N events (%)**	**N events (%)**	**Crude HR (95% CI)**	***P* value**	**Not included in analysis**
ITT*	17 (68)	22 (96)	0·51 (0.27—0.96)	0.037	1
Per protocol	16 (70)	21 (95)	0.55 (0.29—1.06)	0.072	4
Modified ITT	16 (67)	22 (96)	0.49 (0.26—0.94)	0.032	2

Data are mean (95% CI) or n (%) unless otherwise specified

ITT Intention-to-treat, *HR* Hazard ratio

* One participant was excluded from this analysis as this participant died before day 20 and the primary outcome was defined as having to occur day 20–365

Table 3 Secondary outcomes measurements

	Antibiotic group ($n=26$)	Control group ($n=23$)	*P* value	Number missing
Days alive and out of hospital from day 20 to day 365				0
Parametric analysis, mean (95% CI)	315 (281—348)	288 (246—329)	0.31	
Non-parametric analysis, median (IQR)	343 (336—345)	325 (296—345)	0.31	
Death within 365 days, n (%)	2 (8)	5 (22)	0.23	0
Microbial cure at l day 90, n (%)	11 (42)	5 (22)	0.14	0
Clinical cure at day 14, n (%)	18 (78)	8 (38)	0.013	5
Decrease ≥ 200 mL FEV1 from day 0 to 90, n (%)	0 (0)	0 (0)	1.00	6
Admissions with exacerbations within 365 days, mean (95% CI)	1.1 (0.6—1.7)	2.9 (1.3—4.5)	0.037	2
Days on NIV or mechanical ventilation within 90 days, mean (95% CI)	0.04 (-0.04—0.12)	0.13 (-0.02—0.28)	0.27	0
Change in CAT from baseline to 90, mean (95% CI)	-3.9 (-6.3—-1.5)	-2.7 (-5.1—0.2)	0.33	6
Change in BMI from baseline to day 90, mean (95% CI)	-0.3 (-0.7—0.2)	-0.9 (-1.8—0.0)	0.36	6
Change in FEV1 from baseline to day 90, mean (95% CI)	0.07 (-0.03—0.18)	0.01 (-0.04—0.05)	0.20	6

Data are mean (95% CI) or n (%) unless otherwise specified

COPD Chronic obstructive pulmonary disease, *NIV* Non-invasive ventilation, *CAT* COPD assessment test, *BMI* Body mass index, *FEV_1* Forced expiratory volume the first second

anti-pseudomonal antibiotic group, although this did not reach statistical significance (mean 315 days (95% CI 281–348) in the antibiotic group vs. 288 days (95% CI 246–329) in the control group, $p=0.31$; Table 3 and Fig. 3 in Additional file 1). *Death within 365 days from randomisation* occurred in two outpatients (8%) in the dual-systemic anti-pseudomonal antibiotic group and five outpatients (22%) in the control group ($p=0.23$; Table 3 and Fig. 4 in Additional file 1).

The *number of admissions with exacerbation within 365 days from randomisation* was different: mean 1.1 (95% CI 0.6–1.7) in the antibiotic group versus 2.9 (95% CI 1.3–4.5) in the control group, $p=0.037$, Table 3 and Fig. 5 in Additional file 1. Further, *clinical cure at day 14* was 78% in the antibiotic group versus 38% in the control group, $p=0.013$; Table 3.

There was no difference between the two study groups in the remaining secondary outcomes (Table 3); *The mean number of days with non-invasive ventilation or invasive ventilation within 90 days from randomisation* was 0.04 (95% CI -0.04–0.12) in the antibiotic group compared to 0.13 (95% CI -0.02–0.28) in the control group ($p=0.27$), and the occurrence of *microbiological cure at day 90* was 42% of the outpatients in the antibiotic group compared to 22% in the control group ($p=0.14$). The number of patients with *P. aeruginosa*-positive sputum samples according to each study visit are displayed in Table 2 in Additional file 1. We did not detect any significant *change in CAT scores, BMI, or FEV_1 from randomisation to day 90, nor a decrease of ≥ 200 ml in FEV_1 from randomisation to day 365.* However, as illustrated in Fig. 6 in Additional file 1, there was an apparent trend towards decreased CAT score, increased BMI, and increased FEV_1 from randomisation to day 30 in the antibiotic group. The association was explored in a post-hoc analysis and was statistically significant for FEV_1 (Table 3 in Additional file 1). Moreover, there were few adverse effects,

and no severe adverse effect associated to the intervention, in the study population (Table 4 in Additional file 1). Due to the slow progression in recruiting patients during the COVID-19 pandemic lockdown, the trial was stopped prematurely in February 2022 (see "Methods").

Discussion

We conducted a multicenter, randomised, controlled, open-label trial to evaluate the efficacy of dual systemic anti-pseudomonal antibiotics in outpatients with pre-existing lung disease and a recent respiratory tract culture sample with *P. aeruginosa.* We found that the risk of prednisolone or antibiotic requiring exacerbation or death within one year was reduced to about half. The total number of hospitalisation-requiring exacerbations within one year was reduced from almost three to approximately one, and clinical cure at day 14 also improved markedly. In all other secondary outcomes, we observed a non-significant trend in the direction of benefit from the dual systemic antibiotic intervention. These included: *i)* days alive and without hospitalisation from day 20 to 365 from randomisation, *ii)* death from all causes within 365 days, *iii)* number of days with non-invasive ventilation or invasive ventilation within 90 days, *iv)* microbiological cure at day 90, *v)* change in CAT score to day 90, *vi)* change in BMI to day 90, *vii)* change in FEV_1 to day 90, and *viii)* decrease of $\geq$ 200 ml in FEV_1 from randomisation to day 365. No secondary outcomes trended towards harm from the intervention. The majority of outpatients had COPD, followed by non-CF bronchiectasis. The main result seemed to be preserved both among *P. aeruginosa*-naïve and *P. aeruginosa*-non-naïve patients.

To our knowledge, this is the first randomised controlled trial to explore the clinical effects of systemic antibiotic treatment targeting *P. aeruginosa* in patients with pre-existing lung disease and frequent exacerbations. Previously, a smaller and retrospective observational study conducted between 2004–2010 assessed the effects of different antibiotic regimens after the first colonisation of *P. aeruginosa*in 30 patients with non-CF bronchiectasis, in whom the majority were treated with systemic antibiotics for two weeks. Exacerbation frequencies seemed lower after antibiotic treatment [6].

In the past two decades, a growing number of trials have investigated the potential clinical advantages of inhaled antibiotic as eradication treatment in patients with lung disease and recurrent isolation of *P. aeruginosa*. Recent meta-analyses have highlighted some controversy regarding their impact on exacerbations in non-CF bronchiectasis [13, 14]. To date, no randomised controlled trials have tested inhaled antibiotics in patients with asthma or COPD.

Suggestions for combination treatment for *P. aeruginosa* are based on in vitro data [15] and observational studies [8]. Due to important limitations concerning study design and sample size, no clear evidence of the benefits of combination therapy over monotherapy for *P. aeruginosa*bacteraemia has been proposed [16, 17].

Our study was stopped prematurely based on the collapse of recruitment during the COVID-19 pandemic. This decision was made by the trial Steering Committee without any knowledge of the data. The appointed Data and Safety Monitoring Board endorsed the decision. The premature halt of the trial is disappointing, however, our data remains the only trial data on this important question, and further, they are supported by observational studies and microbiological evidence. They do, in fact, inform on a clinical subject to which clinical practice is highly differing worldwide. The signal of the results seems very strong, and importantly, is consistent between the primary outcome and secondary outcomes, and there was 100% follow-up on all outcomes.

Our study's relatively low bacterial eradication rate is in line with findings in previous trials of combined inhaled and systemic antibiotic treatment in non-CF bronchiectasis [5, 18]. Our observations are not surprising as *P. aeruginosa*is known to grow persistently in the airways of patients with chronic pulmonary diseases [3, 19–24]. This was also demonstrated in a sub-study of the present trial, in which we conducted a whole-genome-sequencing on the systematically collected sputum samples in the initial 23 outpatients. This analysis revealed that subsequent growth of *P. aeruginosa* was common, with 83% experiencing it during the 365-day follow-up period. Furthermore, the recurrent *P. aeruginosa*-positive sputum samples harbour the same *P. aeruginosa*clone as the first sputum culture at recruitment [25]. Thus, using dual systemic anti-pseudomonal antibiotic therapy is unlikely to provide a sufficient long-term eradication in patients with pre-existing lung disease. Consequently, the term *eradication therapy* used for dual systemic antibiotics, should be avoided. However, there seems to be possible important clinical short-term effects in terms of a higher clinical cure rate as well as patient reported symptoms (CAT score) and improvement in FEV_1following antibiotic treatment. To our best knowledge, similar improvements in pulmonary function have not been observed in any previous trials assessing inhaled anti-pseudomonal antibiotics in non-CF bronchiectasis [14, 15].

Our trial has limitations apart from the premature closure. Second, we used an unblinded intervention. Prior to study start, the trial steering committee discussed the possibility for double blinding, but the investigators from the sites found it highly unfeasible to convince the department heads to allow admission for until 14 days,

just to receive placebo. Thus, despite the multicenter and randomised study design, this could have affected the assessment of some outcomes, including clinical cure assessment, which was symptom-based and not quantified.

In conclusion, dual systemic antibiotic treatment against *P. aeruginosa* markedly improved critical clinical outcomes like exacerbations in outpatients with COPD, non-CF bronchiectasis or asthma with no clinical reason for admission. Our study thus demonstrates, the severe limitation of premature closure held in mind, that dual antibiotics for two weeks in outpatients with COPD, non-CF bronchiectasis or asthma who have a culture sample with *P. aeruginosa,* and who are not judged as in clinical need of hospitalisation, is well-tolerated and leads to substantially better clinical outcomes within one year. Such an intervention should be considered in patients like the ones included in our trial.

Abbreviations

BMI	Body mass index
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CT	Computer tomography
DSMB	Data and safety monitoring board
FEV1	Forced expiratory volume the first second
GCP	Good clinical practice
HR	Hazard ratio
MRC	Medical research council
NIV	Non-invasive ventilation
Non-CF	Non-cystic fibrosis
ITT	Intention-to-treat

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-024-02860-9.

Additional file 1: 1. Trial group. 2. Study timeline. 3. Study protocol. 4. Statistical analysis plan. 5. Randomisation and masking. 6. Adherence to treatment. 7. Protocol amendment log. 8. Data and Safety Monitoring Board (DSMB) charter. 9. Patient recruitment by trials site. 10. DSMB endorsement letter for early trial termination. 11. Note to statistical analysis plan. 12. Supplemental tables and figures.

Acknowledgements

This trial was investigator initiated. No commercial sponsors were involved in the design and undertaking of the study. The trial investigators have the rights to the results. We thank participating patients, clinical staff, and research staff at all trial sites for contributing to the study.

Authors' contributions

The study director JUSJ and coordinating investigator JE had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JUSJ, JE, PS, TW and NS generated the hypothesis for the study. JE and JUSJ wrote the initial protocol, obtained funding and study approval, and initiated the study. JE, IAA, KA, TSL, AB, RHO, ZBH, JJ, MM, UMW, JLK, JV and CSF recruited and followed the participants. AJ and TWK performed the statistical analyses. JE and IAA coordinated the trial and were responsible for monitoring. All authors of the manuscript contributed to the conception of the study, or the acquisition and interpretation of the data; and critically revised the manuscript. All authors of the manuscript read and approved the final manuscript.

Funding

Open access funding provided by Copenhagen University This work was supported by the Danish Council for Independent Research. The funder has no role in the study design or in the collection, analysis, or interpretation of data, nor in the writing or publication of the paper.

Availability of data and materials

Requests for data, including a study protocol, should be sent to the principal investigator, who will review the request with the TARGET ABC steering committee. If the hypothesis of the request is within the informed consent granted by study participants at the time of inclusion, and the hypothesis is judged to be valid, a data transfer agreement will be prepared in agreement with national legislation for data sharing.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from patients before randomisation. The study was approved by the Ethics Committees (H-15010949), the Danish Medicines Agency (EudraCT 2015–003399-58) and the Danish Data Protection Agency (HGH-2017–036, I-Suite nr 05548) and was monitored according to Good Clinical Practice (GCP) by the GCP unit of the Capital Region of Denmark.

Consent for publication

Not relevant.

Competing interests

The first and last author have no conflicts of interest. CSU reports personal fees from AstraZeneca, GlaxoSmithKline, TEVA, Novartis, Boehringer Ingelheim, TFF Pharmaceuticals, Berlin Chemie, Orion Pharma, Sanofi, Takeda, Pfizer and Chiesi outside of the submitted work. All other authors declare no competing interests.

Author details

[1]Department of Internal Medicine, Herlev Gentofte University Hospital, Section of Respiratory Medicine, Copenhagen University Hospital, Herlev Gentofte, Hellerup, Denmark. [2]Department of Respiratory Medicine and Infectious Diseases, Copenhagen University Hospital, Bispebjerg Frederiksberg, Denmark. [3]Department of Respiratory Medicine, Antwerp University Hospital, Antwerp, Belgium. [4]Laboratory of Experimental Medicine and Pediatrics, University of Antwerp, Antwerp, Belgium. [5]Department of Respiratory Medicine and Infectious Diseases, Copenhagen University Hospital, North Zealand, Denmark. [6]Department of Respiratory Medicine, Copenhagen University Hospital, Hvidovre, Denmark. [7]Department of Respiratory Medicine, Aalborg University Hospital and Department of Clinical Medicine, Aalborg, Denmark. [8]Department of Internal Medicine, Hospital of Southwest Jutland, Esbjerg, Denmark. [9]Department of Clinical Microbiology, Copenhagen University Hospital, Herlev, Denmark. [10]Department of Clinical Microbiology, Copenhagen University Hospital, Hvidovre, Denmark. [11]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark.

Received: 6 February 2024 Accepted: 29 May 2024
Published online: 06 June 2024

References

1. Garcia-Clemente M, de la Rosa D, Máis L, et al. Impact of *Pseudomonas aeruginosa* Infection on Patients with Chronic Inflammatory Airway Diseases. J Clin Med. 2020;24(9):3800.
2. Martines-García MA, Rigau D, Barrecheguren M, et al. Long-Term Risk of Mortality Associated with Isolation of *Pseudomonas aeruginosa* in COPD: A Systematic Review and Meta-Analysis. Int J Chron Obstruct Pulmon Dis. 2022;16(17):371–82.
3. Finch S, McDonnell MJ, Abo-Leyah H, Aliberti S, Chalmers JD. A Comprehensive Analysis of the Impact of Pseudomonas aeruginosa Colonisation on Prognosis in Adult Bronchiectasis. Ann Am Thorac Soc. 2015;12:1602–11.

4. Polverino E, Goeminne PC, McDonnell MJ, et al. European Respiratory Society guidelines for the management of adult bronchiectasis. Eur Respir J. 2017;9(50):1700629.
5. Orriols R, Hernando R, Ferrer A, Terradas S, Montoro B. Eradication Therapy against Pseudomonas aeruginosa in Non-Cystic Fibrosis Bronchiectasis. Respiration. 2015;90:299–305.
6. White L, Mirrani G, Grover M, Rollason J, Malin A, Suntharalingam J. Outcomes of Pseudomonas eradication therapy in patients with non-cystic fibrosis bronchiectasis. Respir Med. 2012;106:356–60.
7. The Global Initiative for Chronic Obstructive Lung Disease. From the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD). 2023. https://www.goldcopd.org/Guidelines/guidelines-resources.html.
8. Hilf M, Yu VL, Sharp J, Zuravleff JJ, Korvick JA, Muder RR. Antibiotic therapy for Pseudomonas aeruginosa bacteremia: outcome correlations in a prospective study of 200 patients. Am J Med. 1989;87:540–6.
9. The Global Initiative for Chronic Obstructive Lung Disease. From the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD). 2017. https://www.goldcopd.org/Guidelines/guidelines-resources.html.
10. Groenewegen KH, Wouters EF. Bacterial infections in patients requiring admission for an acute exacerbation of COPD; a 1-year prospective study. Respir Med. 2003;97:770–7.
11. Ko FW, Ip M, Chan PK, et al. A 1-year prospective study of the infectious etiology in patients hospitalised with acute exacerbations of COPD. Chest. 2007;131:44–52.
12. Miravitlles M, Espinosa C, Fernández-Laso E, Martos JA, Maldonado JA, Gallego M. Relationship between bacterial flora in sputum and functional impairment in patients with acute exacerbations of COPD. Study Group of Bacterial Infection in COPD. Chest. 1999;116:40–6.
13. Laska IF, Crichton ML, Shoemark A, Chalmers JD. The efficacy and safety of inhaled antibiotics for the treatment of bronchiectasis in adults: a systematic review and meta-analysis. Lancet Respir Med. 2019;7:855–69.
14. Tejada S, Campogiani L, Solé-Lleonart C, et al. Inhaled antibiotics for treatment of adults with non-cystic fibrosis bronchiectasis: A systematic review and meta-analysis. Eur J Intern Med. 2021;90:77–88.
15. Giamarellou H. Prescribing guidelines for severe Pseudomonas infections. J Antimicrob Chemother. 2002;49:229–33.
16. Hu Y, Li L, Li W, et al. Combination antibiotic therapy versus monotherapy for Pseudomonas aeruginosa bacteraemia: a meta-analysis of retrospective and prospective studies. Int J Antimicrob Agents. 2013;42:492–6.
17. Paul M, Benuri-Silbiger I, Soares-Weiser K, Leibovici L. Beta lactam monotherapy versus beta lactam-aminoglycoside combination therapy for sepsis in immunocompetent patients: systematic review and meta-analysis of randomized trials. BMJ. 2004;20(328):668.
18. Bilton D, Henig N, Morrissey B, Gotfried M. Addition of inhaled tobramycin to ciprofloxacin for acute exacerbations of Pseudomonas aeruginosa infection in adult bronchiectasis. Chest. 2006;130:1503–10.
19. Valderrey AD, Pozuelo MJ, Jiménez PA, Maciá MD, Oliver A, Rotger R. Chronic colonization by Pseudomonas aeruginosa of patients with obstructive lung diseases: cystic fibrosis, bronchiectasis, and chronic obstructive pulmonary disease. Diagn Microbiol Infect Dis. 2010;68:20–7.
20. Martínez-Solano L, Macia MD, Fajardo A, Oliver A, Martinez JL. Chronic Pseudomonas aeruginosa infection in chronic obstructive pulmonary disease. Clin Infect Dis. 2008;47:1526–33.
21. Murphy TF, Brauer AL, Eschberger K, et al. Pseudomonas aeruginosa in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2008;177:853–60.
22. Rakhimova E, Wiehlmann L, Brauer AL, Sethi S, Murphy TF, Tümmler B. Pseudomonas aeruginosa population biology in chronic obstructive pulmonary disease. J Infect Dis. 2009;200:1928–35.
23. Rossi E, La Rosa R, Bartell JA, Marvig RL, Haagensen JAJ, Sommer LM, et al. Pseudomonas aeruginosa adaptation and evolution in patients with cystic fibrosis. Nat Rev Microbiol. 2021;19:331–42.
24. Marvig RL, Sommer LM, Molin S, Johansen HK. Convergent evolution and adaptation of Pseudomonas aeruginosa within patients with cystic fibrosis. Nat Genet. 2015;47:57–64.
25. Eklöf J, Misiakou MA, Sivapalan P, et al. Persistence and genetic adaptation of Pseudomonas aeruginosa in patients with chronic obstructive pulmonary disease. Clin Microbiol Infect. 2022;28:990–5.

Publisher's Note

Additional File:

TARGETed AntiBiotiCs (TARGET-ABC) multicenter, randomized, controlled, open-label trial

Authors:

Josefin Eklöf MD, Imane Achir Alispahic, Karin Armbruster, Therese Sophie Lapperre, Andrea Browatzki, Rikke Holmen Overgaard, Zitta Barrella Harboe, Julie Janner, Mia Moberg, Charlotte Suppli Ulrik, Helle Frost Andreassen, Ulla Møller Weinreich, Jakob Lyngby Kjærgaard, Jenny Villadsen, Camilla Sund Fenlev, Torben Tranborg Jensen, Christina Wellendorph Christensen, Jette Bangsborg, Christian Ostergaard, Khaled Saoud Ali Ghathian, Alexander Jordan, Tobias Wirenfeldt Klausen, Thyge Lynghøj Nielsen, Torgny Wilcke, Niels Seersholm, Pradeesh Sivapalan, Jens-Ulrik Stæhr Jensen.

THE TARGET-ABC TRIAL GROUP

Study director: Jens Ulrik Stæhr Jensen, Herlev and Gentofte University Hospital

Principal investigator: Josefin Eklöf, Herlev and Gentofte University Hospital

Steering Committee: Jens-Ulrik Stæhr Jensen, Jørgen Vestbo, Julie Janner, Mia Moberg, Therese Lappere, Charlotte Suppli Ulrik, Torgny Wilcke, Kristoffer Marså, Pia Thaning, Helle Frost Andreassen, Ulla Møller Weinreich, Andrea Browatzki, Uffe Bødtger, Ingrid Titlestad, Elisabeth Bendstrup Christian B. Laursen Thomas Benfield, Thomas Benfield Christian Meyer and Merete Raaschou.

Data Safety and Monitoring Board: Tobias Wirenfeldt Klausen, Tor Biering-Sørensen, Philipp Schuetz

GCP unit — Research: Lotte Lund, Sanne Andersen, Charlotte Calov

Trial statisticians: Tobias Wirenfeldt Klausen, Alexander Jordan

Trial site investigators and research staff

Herlev and Gentofte University Hospital: Karin Armbruster, Niels Seersholm, Jon Torgny Wilcke, Pradeesh Sivapalan, Imane Achir Alispahic, Rikke Holmen Overgaard, Jenny Villadsen, Jakob Lyngby Kjærgaard, Christina Wellendorph Christensen, Jette Bangsborg.

Bispebjerg University Hospital: Therese Sofie Lapperre, Helle Frost Andreassen

Hospital of Southwest Jutland: Torben Tranborg Jensen, Tilde Ellingsgaard

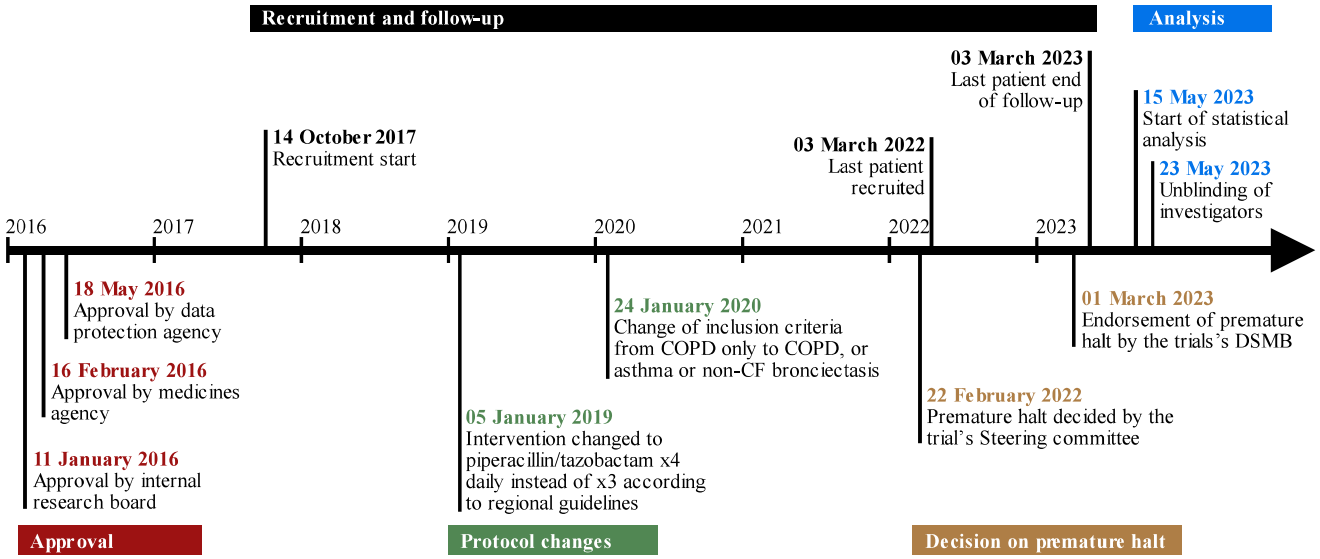
Odense University Hospital: Sofie Lock Johansson

North Zealand University Hospital: Rikke Holmen Overgaard, Camilla Fenlev, Andrea Browatzki, Zitta Barrella Harboe, Thyge Lynghøj Nielsen

Amager and Hvidovre University Hospital: Julie Janner, Mia Moberg, Marie Heidemann, Charlotte Suppli Ulrik, Christian Ostergaard, Khaled Saoud Ali Ghathian,

Aalborg University Hospital: Ulla Møller Weinreich, Roxanna Vijdea

STUDY TIMELINE



STUDY PROTOCOL

Targeted AntiBiotics for Chronic pulmonary diseases (TARGET-ABC):

Can targeted antibiotic therapy improve the prognosis of *Pseudomonas aeruginosa* infected patients with chronic pulmonary obstructive disease, non-cystic fibrosis bronchiectasis or asthma?

A multicenter, randomised, controlled, open-label trial

Scientific Project Sponsor
Chronic Obstructive Pulmonary Disease Trial Network (COP:TRIN) - a network of independent COPD research in Denmark
Chair: Jens-Ulrik Stæhr Jensen, MD, PhD, Associate Professor

Coordinator Investigator
Josefin Eklöf, MD, PhD student, Department of Internal Medicine, Section of Respiratory Medicine, Herlev and Gentofte University Hospital, Copenhagen, Denmark

Principal Supervisor
Jens-Ulrik Stæhr Jensen, MD, PhD, Associate Professor Sponsor, Department of Internal Medicine, Section of Respiratory Medicine, Herlev and Gentofte University Hospital, Copenhagen, Denmark

Co-supervisors
Torgny Wilcke, MD, PhD, Associate Professor, Department of Internal Medicine, Section of Respiratory Medicine, Herlev and Gentofte University Hospital, Copenhagen, Denmark

Niels Seersholm, MD, DMSc, Department of Internal Medicine, Section of Respiratory Medicine, Herlev and Gentofte University Hospital, Copenhagen, Denmark

1. Background

COPD, non-CF bronchiectasis, and asthma are common chronic pulmonary diseases and important causes of death and disability worldwide [1,2,3]. These diseases are characterized by shared common symptoms such as productive cough and susceptibility to recurrent exacerbations that are often associated with infections. These exacerbations lead to accelerated loss of lung function, reduced quality of life, and increased morbidity and mortality and have major socio-economic consequences [4,5].
Compared to COPD and asthma, which both are diagnosed on the basis of airflow obstruction and therefore are physiological diagnoses, bronchiectasis is a structural diagnosis with the presence of permanent airway dilatation on radiological imaging [4,5,6]. However, the co-existence of bronchiectasis and asthma or COPD is common [7].
Pseudomonas aeruginosa [8] has been reported to be present in the lower airways in up to 20% of patients with COPD [9,10,11] and is frequently detected in patients with non-CF bronchiectasis [12]. The bacterium has also been observed in patients with asthma [13]. Nevertheless, the influence of *P. aeruginosa* on the progression of these diseases is far from fully elucidated. The bacterium is seen primarily in advanced diseases with severely impaired lung function [12,13,14] and is associated with increased frequency of exacerbation, prolonged hospitalization, and poor long-term prognosis with increased mortality rates compared to *P. aeruginosa*-negative patients [15].
However, since an impairment of lung function itself is a strong predictor of morbidity and mortality, it is not certain whether infection with *P. aeruginosa* is secondary to lung function impairment or whether the presence of *P. aeruginosa* itself leads to pulmonary tissue inflammation and remodeling, impaired lung function, and overall poor prognosis.

Thus, the role of *P. aeruginosa* on the progression of COPD, non-CF bronchiectasis, and asthma is poorly characterized. To date, evidence-based guidelines for the management and treatment of *P. aeruginosa* infection are lacking, and the management of *P. aeruginosa* is often based on expert consensus and studies of other chronic lung diseases, including CF. In CF, *P. aeruginosa* is a leading cause of morbidity and early death with evidence of improved clinical outcomes through aggressive and targeted antibiotic treatment [16]. In Denmark, the first treatment choice for clinically treatment-requiring *P. aeruginosa* infection is usually 10–14 days of antibiotic combination therapy with intravenous piperacillin/tazobactam and oral ciprofloxacin [17].

With this randomized controlled trial, we aim to increase the understanding of the clinical significance and consequences of *P. aeruginosa* infection in patients with chronic, non-CF pulmonary disease. The main purpose is to investigate if targeted, antibiotic treatment of *P. aeruginosa* improves the disease prognosis in patients with exacerbation of COPD, non-CF bronchiectasis or asthma, and *P. aeruginosa*-positive lower respiratory tract culture sample.

2. Aim and hypothesis

The aim of this study is to investigate whether antibiotic treatment for *P. aeruginosa* can improve prognosis in patients with COPD, non-CF bronchiectasis or asthma. Our hypothesis is, that antipseudomonal antibiotics improve prognosis by reducing exacerbations.

3. Method

3.1 Design

The study is a multicenter, randomized, controlled, open-label trial in outpatients with COPD, non-CF bronchiectasis, and asthma with current *P. aeruginosa*-positive lower respiratory tract sample. Study participants are followed for 1 year. Participants are recruited by investigators who are employed at the participating respiratory outpatient clinics in Denmark.

Patients will be randomised 1:1 to one of the two study groups:

I. Intervention group: antibiotic treatment (administered in-hospital)
II. Control group: no antibiotic treatment

The first choice of antibiotic treatment is dual therapy with intravenous piperacillin/tazobactam 4 /0,5 gram 4 times daily and oral ciprofloxacin 500 mg 2 times daily for 14 days. In case of penicillin allergy or antibiotic resistance, intravenous piperacillin/tazobactam is replaced by intravenous ceftazidime or meropenem. In case of fluoroquinolone allergy or antibiotic resistance, intravenous beta-lactam is given as monotherapy.

3.2 Randomisation

Randomisation is conducted using a secure web application (REDCap; www.projectredcap.org) where inclusion and exclusion criteria are required to be filled out correctly in order to randomise a study participant. Pre-stratified block randomisation with blocks of varying and blinded size is applied to ensure equal distribution of patients in the study groups based on study center (respiratory outpatient clinic) and age (above or below 70 years of age).

3.3 Enrolment and Inclusion

All consecutive outpatients with COPD, non-CF bronchiectasis, or asthma and current *P. aeruginosa*-positive respiratory sample from the participating study centres (respiratory departments) are considered for study enrollment. Patients are invited to participate in the trial if they fulfil the following inclusion and exclusion:

3.4 Inclusion criteria:

- *P. aeruginosa*-positive lower respiratory tract sample
- COPD, non-CF bronchiectasis, or asthma verified by a respiratory specialist based on clinical assessment and additional tests:
  a) COPD: spirometry
  b) Asthma: reversibility
  c) Non-CF bronchiectasis: high-resolution computed tomography scan
- Minimum of two previous exacerbations, or one previous hospitalization-requiring or emergency room-demanding exacerbation, with the treatment of systemic prednisolone and/or antibiotics within the last 12 months
- Written informed consent

3.5 Exclusion criteria:

- Immunomodulating treatment (except ≤ 10 mg prednisolone/day)
- Men < 40 years
- Women ≤ 55 years
- Non-menopausal women > 55 years (i.e., menstruation within the last 12 months)
- Life expectancy < 90 days
- Severe mental illness or severe linguistic problem
- Known drug allergy to (i) fluroquinolone and (ii) both penicillin/piperacillin, cephalosporin, and carbapenems
- ≥ 2 previous eradication attempts of *P. aeruginosa* within the last 12 months or 1 completed within the last 14 days *
- Patients who clinically require hospitalization and anti-pseudomonal antibiotic treatment. This exclusion criterion must be discussed with the coordinating investigator before the final decision on exclusion is made

*Defined as 10-14 days of dual therapy with beta-lactam and fluoroquinolone.

4. Data collection

The daily project management is carried out by the primary investigators and sub-investigators, consisting of health professionals from the departments involved in the trial, and is coordinated by the coordinating investigator (Josefin Eklöf). Data is collected in electronical case report forms in Redcap, specific to each participant., and include demographic data, health status, hospitalisations, clinical parameters, study results and prescribed medication are recorded. Case report forms are archived for 15 years. Collection and storage of data is in compliance with Good Clinical Practice (GCP) guidelines and is regularly monitored by local GCP units.

Study overview is summarised in Table 1. Follow-up visits are scheduled after 14, 30, 60, 90 and 365 days. Blood samples are taken at the start of the study and before administration of any antibiotics, after 14 days and continuously every 3 days during antibiotic treatment. Antibiotic therapy is started according to the current guidelines for each preparation, incl. adjustment regarding the participant's usual medication, kidney function, age, side effects and possibly allergies. Administration of antibiotics is registered and recorded using the department's applicable electronic medicine module. HRCT (high-resolution computed tomography) is performed at the start of the study in order to identify underlying prevalence of emphysema and bronchiectasis.

Table 1. Study overview

Study period							
	Enrolment	**Intervention**	**Follow-up**				
Visit number	**1**		**2**	**3**	**4**	**5**	**6**
Study day	**0**	**0-14**	**14**	**30**	**60**	**90**	**365**
Enrolment							
Eligibility screening	X						
Informed consent	X						
Randomisation	X						
Study arm							
Intervention group: antibiotic treatment, in-hospital		X					
Control group: no antibiotic treatment, not hospitalised							
Data collection and examinations							
Demographics	X		X				
Sputum sample	X		X	X	X	X	X
Body mass index (BMI)	X		X	X	X	X	X
Medical Research Council Dyspnoea Scale (MRC)	X		X	X	X	X	X
COPD Assessment Test (CAT)	X		X	X	X	X	X
Spirometry	X		X	X	X	X	X
Vital parameters	X		X				
High resolution computed tomography (HRCT)	X						
Blood samples	X	X	X				

5. Research biobank

A research biobank with blood and sputum samples collected at enrolment and follow-up visits have been established. This biobank will be used for future research, including genomic analyses of *P. aeruginosa*-positive sputum samples collected during the study. Separate information and consent material for the study participants have been prepared and the Danish Science Ethics Committee and the Danish Data Protection Agency. The samples are stored in an anonymously form for 15 years. The material is reserved for the current research study and can only be used for other research projects with the permission from the Danish Science Ethics Committee.

6. Statistical considerations and power sample calculation

Data will be analyzed using intention-to-treat (ITT) principles, including all the data available, regardless of whether the intervention was completed or not. The aim of the ITT analysis is also to provide unbiased comparisons among the two study groups and to avoid the effects of potential study dropouts and protocol deviations.

Patients in the control group will be compared to patients in the intervention group. We will use Fisher's exact test and chi-squared test for dichotomous outcomes and *T*-test for continuous outcomes. The timed dichotomous outcomes will be visualized through Kaplan-Meier plots. Furthermore, adjusted analyses will be performed with a multivariable Cox proportional hazards model, adjusting for baseline variables and calculating hazard ratios. Data will be processed and analyzed in SAS and graphs are generated in Microsoft Excel and other graph programs.

The sample size is calculated based on 80% power, a two-sided 5% significance level and the following estimates and indicative figures for COPD:

1) *P. aeruginosa* incidence 5-20%
2) 67% of study participants in the antibiotic-free group have exacerbated or died within 12 months
3) 47% of study participants in the antibiotic group have exacerbated or died within 12 months

Thus, we expect an effect size of 20% absolute reduction (30% relative reduction) of exacerbation or mortality in the antibiotic group. Based on the above, a total of 150 patients should be included. Furthermore, to avoid error estimates and risk of including too few patients (underpowering), the study is "event driven" and will only be closed when at least 67% of patients in the antibiotic-free group have exacerbated or died, but twelve months must have passed, also if more than 67% of patients have experienced the primary outcome event.

For non-CF bronchiectasis and asthma:

1) Annual exacerbation rate with *P. aeruginosa*: 2.85
2) Annual exacerbation rate without *P. aeruginosa*: 1.80
3) Standard deviation, annual exacerbation rate: 1.5

Based on these reference estimates [18], a total of 66 patients should be included (i.e., 33 patients in each study group). Since most study participants potentially could be COPD patients, the number from the COPD sample size calculation is used in order not to risk underpowering the study. If non-COPD patients are recruited, the power will thus be increased to > 80%.

In cases of low *P. aeruginosa* incidence (PAi), patients will need to be recruited from several pulmonary departments to achieve the desired sample size, as calculated below. The figures are based on estimation of approximately 2,000 patients with GOLD Class C or D/outpatient clinic. Of these, 1/3 are expected to be able to produce sputum sample. However, the dropout rate is estimated to be about the same level, thus there are approximately potentially 400 patients/outpatient clinic/year.

5% PAi: 0.05 x 400 = 20 patients/outpatient clinic/year x 8 outpatient clinics = 160 patients / year

10% PAi: 0.10 x 400 = 40 patients/outpatient clinic/year x 4 outpatient clinics= 160 patients/year

15% PAi: 0.15 x 400 = 60 patients/outpatient clinic/year x 3 outpatient clinics= 180 patients/year

20% PAi: 0.20 x 400 = 80 patients/outpatient clinic/year x 2 outpatient clinics= 160 patients/year

An independent Data Safety Monitoring Board will monitor the safety of the trial by conducting interim analyses based on primary and secondary endpoints when half of the study population (i.e. 75 patients) has completed the study.

6.1 Primary outcome

- Time to prednisolone and/or antibiotic requiring exacerbation, in primary or secondary health care sector, or death from day 20 to day 365 from randomisation *

6.2 Secondary outcomes

- Days alive and without hospitalisation from day 20 to day 365 from randomisation
- Death within 365 days from randomisation
- Number of admissions with exacerbation of the chronic lung disease within 365 days from randomisation
- Number of days with non-invasive-ventilation or invasive ventilation within 90 days from randomisation
- Microbiological cure **
- Clinical cure ***
- Change in COPD Assessment Test (CAT) between baseline and day 90 from randomisation
- Change in BMI between baseline and day 90 from randomisation
- Change in FEV_1 between baseline and day 90 from randomisation
- Decrease of $\geq$ 200 ml in FEV_1 from randomisation to day 365

*The co-primary outcome of "days alive and without hospitalisation from day 20 to day 365 from randomisation" was degraded to the first secondary outcome by the trial statistician in agreement with the coordination investigator and study director since this outcome would be severely underpowered because of the premature closure of the trial. This decision was taken before the database was unblinded to the analysis (see Supplementary page 24).

**Microbiological cure: *P. aeruginosa*-negative sputum culture until day 90. No microbiological cure: positive sputum culture with clonally same *P. aeruginosa* strain $\leq$ day 90. Re-infection: positive sputum sample with non-clonally same *P. aeruginosa* strain $\leq$ day 90.

***Clinical cure: cessation or improvement of clinical signs and symptoms related to *P. aeruginosa* $\leq$ day 14. Clinical failure: persistent or worsening of clinical signs and symptoms related to *P. aeruginosa* $\leq$ day 14.

7. Adverse effects and risks

Treatment with piperacillin/tazobactam and ciprofloxacin are associated with a low frequency of serious side effects (<0.01%). These include pancytopenia, bone marrow depression, psychological reactions and depression, seizures, anaphylactic reaction/shock, renal failure, Steven-Johnson syndrome, toxic epidermal necrolysis, liver necrosis, ventricular arrhythmia and pseudomembranous colitis. The Summary of Product Characteristics (SCP) will be used as a reference document when assessing adverse reactions. As the study is focusing on the long-term clinical effects of the drugs, only unknown adverse reactions not recorded in the SPC will be registered in the trial.

The following events are expected and naturally occurring as part of the underlying lung disease and lung infection and do not need be recorded as adverse events: coughing, respiratory mucus, shortness of breath, wheezing, chest pressure, palpitations, lower leg swelling, unrest, anxiety, sleep disorders, eating disorders, fatigue, dizziness and abnormalities in blood test responses that are related to infection and that are not considered clinically significant. Likewise, exacerbation of COPD, non-CF bronchiectasis or asthma will not be registered as a serious adverse event as occurrence of this is already included as (primary) endpoint, and thus will be assessed by the DSMB in the pre-planned interim analysis. All other serious adverse events or reactions must be immediately reported (= within 24 hours of investigator becoming aware of a serious adverse event or reaction) to the sponsor to assess if a serious related adverse event is unexpected and thus possibly a suspected unexpected serious adverse reactions (SUSAR). All registered adverse events and adverse reactions will be reported at the end of the trial in a final report to the Danish Health Authority. All serious adverse reactions and adverse events must be recorded annually.

Examination by HRCT may cause discomfort in the form of claustrophobia in some patients. In addition, a CT scan involves exposure to rays equal to approximately 5 years of background radiation in Denmark. As the study participants only will be examined once, and as the median life expectancy for this group of patients with

severe COPD is approximately 5 years, the diagnostic benefit it is estimated to be greater than the risk of exposure.

8. Removal from and interruption of the trial
Investigators may interrupt the intervention at any time if there is a medical justification, safety risk or a requirement from the authorities. If the investigator deems it necessary, he or she may exclude the participant from the trial. However, in general, no subject should be removed from the study for a protocol violation prior to confirmation by the coordinating investigator. In addition, a study participant is only to be withdrawn from the study if the participant explicitly asks for withdrawal.

9. Funding
The research project is financed by grants from the Independent Research Fund Denmark (8020-00425B) and the Research committee at Herlev and Gentofte University Hospital and by participating study centres.

10. Access to data
It is the Steering Committee's belief that sharing of knowledge creates more and better scientific results. Requests for sharing data with other groups will be submitted to the Steering Committee. If the hypothesis to be examined has a relevant scientific content, and is not planned to be examined by our group, we will allow the use of our data

11. Publication of study results
The trial registered at clinicaltrials.gov (NCT03262142, August 25, 2017). The data from the TARGET ABC trial will be available once the study is completed. All results will be published in scientific contexts, including international journals, regardless of whether they are positive, negative or in-conclusive, and with authorship according to the Vancouver recommendations.

12. Scientific ethical statement
P. aeruginosa represents a potentially significant cause of exacerbation and mortality in patients with COPD, non-CF bronchiectasis, and asthma. However, the role of *P. aeruginosa* in this setting is poorly characterized, and to date, evidence-based guidelines for management and treatment of *P. aeruginosa* infection are lacking. With this trial, we aim to increase the knowledge of the clinical consequences of antibiotic treatment against *P. aeruginosa* in patients with COPD, non-CF bronchiectasis and asthma.

Using a multicenter, randomized, controlled design, we will allocate 150 patient with COPD, non-CF bronchiectasis or asthma and current *P. aeruginosa*-positive respiratory sample (1:1) to either no antibiotic treatment or 14 days of dual anti-pseudomonal antibiotic therapy. Thus, we seek to create evidence at level 1B to determine whether targeted antibiotic treatment against *P. aeruginosa* can reduce exacerbations in COPD, non-CF bronchiectasis and asthma and thereby improve the prognostic outcome in a group of severe and vulnerable patients with chronic lung disease.

The trial is carried out in accordance to the Declaration of Helsinki and follows Good Clinical Practice. Study methods and statistical analyses have been carefully considered and it is our strongest belief, that the trial will contribute with essential knowledge that will help clinicians to guide future patients towards evidence-based and improved therapeutically strategies. In addition, the likelihood of serious adverse reactions to the antibiotics is expected to be low and the investigator can always interrupt treatment if it is considered contraindicated. Patients who do not wish to participate in the trial and study participants who withdraw their informed consent will be offered treatment according to the standard guidelines at the specific study department. Based on the above considerations, we believe that the trial is ethically sound and can be conducted without exposing the study participants to unjustifiable risks.

13. Protocol references
1. Soriano JB, Abajobir AA, Abate KH, Abera SF, Agrawal A, Ahmed MB, et al. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med. 2017;5(9):691–706.

2. Henkle E, Chan B, Curtis JR, Aksamit TR, Daley CL, Winthrop KL. Characteristics and Health-care Utilization History of Patients With Bronchiectasis in US Medicare Enrollees With Prescription Drug

Plans, 2006 to 2014. Chest. 2018;154(6):1311–20.

3. Jacobs DM, Ochs-Balcom HM, Noyes K, Zhao J, Leung WY, Pu CY, et al. Impact of Pseudomonas aeruginosa isolation on mortality and outcomes in an outpatient chronic obstructive pulmonary disease cohort. Open Forum Infect Dis. 2020;

4. GOLD. Global intiative for Chronic Obstructive Lung Disease 2020 Report: pocket guide to COPD daignosis, management, and prevention, a guide for health care professionals. GOLD 2020. 2020. https://goldcopd.org.

5. Global Initiative for Asthma: 2019 GINA Report. Global strategy for asthma management and prevention. https://ginasthma.org/wpcontent/uploa ds/2019/06/GINA-2019-main-report-June-2019-wms.pdf.

6. Polverino E, Goeminne PC, McDonnell MJ, Aliberti S, Marshall SE, Loebinger MR, et al. European Respiratory Society guidelines for the management of adult bronchiectasis. European Respiratory Journal. 2017.

7. Quint JK, Millett ERC, Joshi M, Navaratnam V, Thomas SL, Hurst JR, et al. Changes in the incidence, prevalence and mortality of bronchiectasis in the UK from 2004 to 2013: A population-based cohort study. Eur Respir J. 2016;47(1):186–93.

8. Tuon FF, Dantas LR, Suss PH, Tasca Ribeiro VS. Pathogenesis of the Pseudomonas aeruginosa Biofilm: A Review. Pathogens. 2022.

9. Garcia-Vidal C, Almagro P, Romaní V, Rodríguez-Carballeira M, Cuchi E, Canales L, et al. Pseudomonas aeruginosa in patients hospitalised for COPD exacerbation: A prospective study. Eur Respir J. 2009;

10. Groenewegen KH, Wouters EFM. Bacterial infections in patients requiring admission for an acute exacerbation of COPD; a 1-year prospective study. Respir Med. 2003;

11. Patel IS, Seemungal TAR, Wilks M, Lloyd-Owen SJ, Donaldson GC, Wedzicha JA. Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations. Thorax. 2002;

12. Borekci S, Halis AN, Aygun G MB. Bacterial colonization and associated factors in patients with bronchiectasis. Ann Thorac Med. 2016;11(1):55–9.

13. Holm JPY, Hilberg O, Noerskov-Lauritsen N, Bendstrup E. Pseudomonas aeruginosa in patients without cystic fibrosis is strongly associated with chronic obstructive lung disease. Dan Med J. 2013;

14. Miravitlles M. Relationship Between Bacterial Flora in Sputum and Functional Impairment in Patients With Acute Exacerbations of COPD. CHEST J. 1999;116(1):40–6.

15. Sangtam N, Haorongbam S, Silpa K, Singh YP. Bronchiectasis in patients with chronic obstructive pulmonary disease in a tertiary care center in North-East India. Int J Adv Med. 2020;

16. Folkesson A, Jelsbak L, Yang L, Johansen HK, Ciofu O, Hoiby N, et al. Adaptation of Pseudomonas aeruginosa to the cystic fibrosis airway: An evolutionary perspective. Nat Rev Microbiol. 2012;10(12):841–51.

17. Guidelines for antibiotic treatement of acute pulmonary infection with Pseudomonas aeruginosa in COPD. Capital Region, Denmark.

18. Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, et al. The bronchiectasis severity index an international derivation and validation study. Am J Respir Crit Care Med. 2014;189(5):576–85.

STATISTICAL ANALYSIS PLAN

Targeted AntiBiotics for Chronic pulmonary diseases (TARGET-ABC)
A multicenter, randomized, controlled, open-label trial

ClinicalTrials.org Identifier: NCT03262142

Author: Josefin Eklöf, Jens-Ulrik Stæhr Jensen

Introduction:

This is a multicenter, randomized, controlled, open-label trial evaluating the effect of antibiotic treatment for *P. aeruginosa* in patients with chronic pulmonary disease.

The aim of the study is to investigate whether targeted antibiotics against *P. aeruginosa* can reduce exacerbations and mortality in patients with chronic obstructive pulmonary disease (COPD), non-CF bronchiectasis (non-CF BE) and asthma.

The patients are enrolled in the trial only after obtaining informed consent. The trial is conducted at seven centers in Denmark:

1. Department of Internal Medicine, Section of Respiratory Medicine, Herlev-Gentofte Hospital, University of Copenhagen.
Primary investigator: Josefin Eklöf, MD, PhD.
2. Department of Pulmonary and Infectious Diseases, University Hospital North Zealand Hospital. Primary investigator: Andrea Browatzki, MD.
3. Department of Respiratory Medicine, Amager-Hvidovre Hospital, University of Copenhagen.
Primary investigator: Julie Janner, MD, PhD.
4. Department of Respiratory Medicine, Bispebjerg-Frederiksberg Hospital, University of Copenhagen.
Primary investigator: Therese Lapperre, MD, PhD, Research Associate Professor.
5. Department of Respiratory Medicine, Aalborg Hospital, University of Aalborg.
Primary investigator: Ulla Weinreich, MD, PhD, Research Associate Professor.
6. Department of Respiratory Medicine, Odense University Hospital.
Primary investigator: Sofie Johansson, MD, PhD
7. Department of Internal Medicine, Section of Respiratory Medicine, Hospital of Southwest Jutland, Esbjerg.
Primary investigator: Torben Tranborg Jensen, MD.

Patients will be randomized 1:1 to one of the two treatment arms:

a) **Intervention group**: intravenous beta-lactam in combination with oral ciprofloxacin for 14 days
b) **Control group**: no antibiotic treatment

The analyses described in this document will be performed by the coordinating investigator, Josefin Eklöf, in cooperation with the scientific sponsor, Jens Ulrik Stæhr Jensen, once the data have been entered, cleaned, and released for use.

This statistical analysis plan provides a detailed description of the statistical analyses that will be performed for the evaluation of the primary and secondary endpoints of the TARGET-ABC study.

The analyses described in this document are compatible with the recommendations of the CONSORT 2010 statement.

Analyses:

Data will be analysed using intention-to-treat (ITT) principles, including all the data available, regardless of whether the participant completed the intervention or not. The aim of the ITT analysis is also to provide unbiased comparisons among the two study groups and to avoid the effects of potential study dropouts and protocol deviations. The primary outcome will also be subject to a modified ITT analysis (in study participants who started but did not complete the intervention) and per protocol analysis (in study participants who completed intervention).

A Consort diagram of participants will be presented in the study.

Patients who withdraw their consent for the use of their data will not be included in any analysis. Patients who merely withdraw consent to the intervention, will be included in the ITT and modified ITT analysis. We will report cases of withdrawal and the study group to which the participant was originally allocated.

All analyses will be performed using SAS software.

Sample size:
The power to avoid type II error is 80% (1-β) at a two-sided 5% significance level. We used a group-sequential design, allowing for one interim analysis at half target recruitment. This provides a sample size of 150 subjects. All confidence intervals reported will be 95% confidence intervals.

Descriptive analyses:
The following baseline characteristics will be presented within each randomized study group:

- Age, years, median (IQR)
- Male sex, n (%)
- Ethnicity (Caucasian, African (incl. Afro-American), Asian, Inuit, Unknown/other), n (%)
- Body mass index (kg/m2), median (IQR)
- Medical Research Council dyspnea scale, n (%)
- Current smoking, n (%)
- Former smoking, n (%)
- Non-smoking, n (%)
- Pack-years tobacco history, median (IQR)
- COPD assessment test score (CAT), median (IQR)
- Support with activities of daily living at home, n (%)
- Increased dyspnea, n (%)
- Increased sputum volume, n (%)
- Increased sputum purulence, n (%)
- Increased cough, n (%)
- Systolic blood pressure (mm Hg), median (IQR)
- Diastolic blood pressure (mm Hg), median (IQR)
- Heart rate, beats/min, median (IQR)
- Oxygen saturation with nasal oxygen, median (IQR)
- Respiratory rate, breaths/min, median (OQR)
- Temperature (°C), median (IQR)

- Exacerbation frequency in previous year, median (IQR)
- Current or former use of respiratory medication, including antibiotics, n (%)
- Use of long-term oxygen therapy, n (%)
- Use of noninvasive mechanical ventilation, n (%)
- Co-morbidities, n (%)
- FEV1, L, median (IQR)
- FEV1 % predicted, median (IQR)
- FVC, L, median (IQR)
- FVC % predicted, median (IQR)
- FEV1/FVC ratio, %, median (IQR)

Follow-up data /missing data

We expect the extent of missing data to be small in the current trial, and we do not expect that any patients will be lost to follow-up for the primary endpoint.
Following measures will be used in case of missing data:

1) For each baseline variable, the percent of any missing values will be reported.
2) The proportion of patients followed for each outcome data parameter will be reported for the predefined primary and secondary outcomes – as well as in any potential exploratory outcome analyses suggested by external reviewers or editors.
3) Characterization participants for whom no outcomes were observed.
4) Report possible reasons for missing outcome data.
5) We will perform sensitivity analysis to quantify the effect of missing outcome data using multiple imputation on study results

Primary objective and outcomes

The primary outcome is *

1) Time to prednisolone and/or antibiotic requiring exacerbation, in primary or secondary health care sector, or death from day 20 to day 365 from randomisation

The primary outcome will also be analysed as an adjusted analysis using a multivariable Cox proportional hazards model, adjusting for the following variables: sex (male vs. female), CAT-score at recruitment (< 21 vs. ≥ 21) and FEV1 % predicted at recruitment (<50% vs. >= 50%).

*The co-primary outcome of "days alive and without hospitalisation from day 20 to day 365 from randomisation" was degraded to the first secondary outcome by the trial statistician in agreement with the coordination investigator and study director since this outcome would be severely underpowered because of the premature closure of the trial. This decision was taken before the database was unblinded to the analysis (see Supplementary page x).

Secondary objective and outcomes

1) Days alive and without hospitalisation from day 20 to day 365 from randomisation
   Analysis: t-test or Mann-Whitney U test

2) Death within 365 days from randomisation
   Analysis: Fisher's exact test or Chi squared test

3) Number of re-admissions with pulmonary exacerbation within 365 days from randomization
   Analysis: t-test or Mann-Whitney U test

4) Number of days with non-invasive-ventilation or invasive ventilation within 90 days from randomization
   Analysis: t-test or Mann-Whitney U test

5) Microbiological cure (defined as *P. aeruginosa*-negative sputum culture until day 90)
   Analysis: Fisher's exact test or Chi squared test

6) Clinical cure day 14 (defined as cessation or improvement of clinical signs and symptoms related to *P. aeruginosa* before or on day 14)
   Analysis: Fisher's exact test or Chi squared test

7) Change in COPD Assessment Test (CAT) from randomization to day 90
   Analysis: ANOVA will be used to analyse the difference between the two means

8) Change in body mass index (BMI) from randomization to day 90
   Analysis: ANOVA will be used to analyse the difference between the two means

9) Change in FEV_1 from randomization to day 90
   Analysis: ANOVA will be used to analyse the difference between the two means

10) Decrease of $\geq$ 200 ml in FEV_1 from randomization to day 365
    Analysis: Fisher's exact test or Chi squared test

Interim Analysis:
The interim analysis will focus on reporting:

i) Baseline characteristics
ii) Primary outcome: Time to prednisolone and/or antibiotic requiring exacerbation or death, in primary or secondary health care sector, from day 20 to day 365 from randomization (using O-Brien-Fleming Plot)
iii) All-cause mortality at 365 days
iv) Microbiological cure
v) Futility assessment. Recruitment rate compared to planned recruitment.

Blinding of the statistician
The detailed analysis plan was written in strict concordance with the trial protocol approved by the regulatory authorities prior to recruitment initiation. The entire statistical analysis plan is published at www.coptrin.dk (before the trial was finalized and while the database was closed). All analyses will be done prior to breaking of the randomization code (analysis comparisons between “arm A” and “arm B”). The coordinating investigator and the study sponsor and principal investigator will conjointly perform all the data analyses according to this plan, except the interim analysis, which will be performed by a statistician who is not an investigator in the trial. An unblinding date will be chosen and published online at www.coptrin.dk and on this date, the allocation will be unblinded. After the unblinding, no further analysis will not be done, except on demand of reviewer or editor during the publication process.

Figures and tables
The first figure will be a Consolidated Standards of Reporting of Randomized Trials (CONSORT) flow chart. The second figure will be a Kaplan-Meier plot to describe the process of death by treatment arms. The first table will be the baseline characteristics of the ITT population. The second table will be of the primary and secondary outcomes according to the two groups and pair-wise comparisons.

Interim and final analysis

The interim analysis was planned at half target recruitment (75 patients), with a focus on reporting data on the primary outcome (time to prednisolone and/or antibiotic requiring exacerbation or death at day 20 to day 365 from randomisation), all-cause mortality at day 365, microbiological cure at day 14 and assessment of the study's futility. An independent data and safety monitoring board (DSMB) was appointed to review the trial's safety, efficacy, and progression (Appendix page 18: DSMB members and charter). Due to slow recruitment rate, the steering committee of the trial decided to stop further recruitment in February 2022, where approximately 1/3 of the planned patients had been enrolled in the study (Appendix page 3: Study timeline). This decision was tested with the DSMB, who endorsed it (Appendix page 23). Due to the considerable reduction of the study size, the primary outcome was conducted solely as a "time to event" analysis, and the "days alive and out of hospital" analysis was degraded from a co-primary outcome to the first secondary outcome (please see under "Outcomes"). Data analyses were performed by an analysis team (TWK and AJ), including a trial statistician (TWK), after the final data from the last patients last follow-up visit was entered and the database was locked. All analyses were done prior to breaking of the randomisation code. The study group was presented to the results and unblinded at a scheduled unblinding-meeting after the analyses were performed.

RANDOMISATION AND MASKING

The randomisation sequence was generated using a computed generator, stratified according to study site and age (≤70 years vs. >70 years). Online inclusion of patients according to the concealed sequence was done with an independent, centralised, 24 hour-available, web-based system (Redcap). The randomisation sequence was prepared by PS, with instructions from the study director (JUSJ), who did not participate in the enrolment of patients in the trial, nor outcome assessment. Since the study was open-label and without a placebo, both investigator, staff and patients were aware of the allocation to either the control arm or antibiotic intervention from the time of randomisation.

ADHERENCE TO TREATMENT

	Antibiotic intervention group, n (percent)
Patients who did not adhere to the study protocol and received no dual systemic antibiotics	1 (3·8)
Patients who were partially adherent and received 1 to 13 days of dual systemic antibiotics	1 (3·8)
Total	2 (7·7)

	Control group, n (percent)
Patients who were partially adherent and received 1 to 13 days of pseudomonas active antibiotics	1 (4·3)
Patients who were fully non-adherent and received 14 days of pseudomonas active antibiotics	0 (0·0)
Total	1 (4·3)

PROTOCOL AMENDMENT LOG

Date	**Description of changes**
2 March 2018	Specification of exacerbation-inclusion criteria: exacerbation of COPD treated with prednisolone or/and antibiotics
5 January 2019	Change of antibiotic intervention. Standard dose regiment of piperacillin/tazobactam changed from three to four times daily according to regional guidelines (9 November 2018 and EUCAST)
21 October 2019	Added Esbjerg Hospital as trial site
24 January 2020	Change of inclusion criteria: Changed from requiring diagnosis with COPD to requiring diagnosis with COPD or/and asthma or/and non-cystic fibrosis bronchiectasis. Change of intervention. Added possibility of home treatment.

DATA AND SAFETY MONITORING BOARD CHARTER

TARGET ABC trial:
Targeted AntiBiotics for Chronic pulmonary diseases
-
A multicenter, randomized, controlled, open-label trial

Study identification information

A. **Sponsors protocol code:** TARGET ABC (ClinicalTrials.org Identifier: NCT03262142)

B. **Study title:** Targeted antibiotics for chronic pulmonary diseases

C. **Sponsor:** Jens-Ulrik Stæhr Jensen, MD, PhD, professor, Department of Internal Medicine, Section of Respiratory Medicine, Copenhagen University Hospital - Gentofte, Hellerup, Denmark.

D. **Coordinating investigator:** Josefin Eklöf, MD, PhD, Department of Internal Medicine, Section of Respiratory Medicine, Copenhagen University Hospital - Gentofte, Hellerup, Denmark.

E. **Study centers:**
1. Department of Internal Medicine, Respiratory Medicine Section, Herlev and Gentofte Hospital, Denmark
2. Department of Respiratory Medicine and Infectious Medicine, North Zealand Hospital, Denmark
3. Department of Respiratory Medicine and Infectious Diseases, Bispebjerg Hospital, Denmark
4. Department of Respiratory Medicine, Amager and Hvidovre Hospital, Denmark
5. Department of Respiratory Medicine, Aalborg Hospital, University of Aalborg
6. Department of Respiratory Medicine, Odense University Hospital.
7. Department of Respiratory Medicine, Southwest Jutland Hospital, Esbjerg, Denmark

F. **Members of the DSMB:**

The following members have been requested to be part of DSMB:

- Tor Biering Sørensen, MD, Ph.d., MPH
    - Dept of Cardiology, Gentofte University Hospital, Hellerup, Denmark
- Philipp Schuetz, Professor, Dr.Med. MPH
    - Kantonspital Aarau AG |
    - KSA · Internal Medicine & Emergency Medicine
- Tobias Wirenfeldt Klausen, MSc, trial statisticians

The independent Data and Safety Monitoring Board (DSMB) is established to ensure the safety of research participants and the integrity of the study data. It will periodically monitor progress, efficacy, safety and other confidential data from this trial. It is composed of experts in relevant medical fields who have no direct relationship with the study. Outcome data will be privileged and shared only with members of the DSMB during the conduct of the trial.

Study Overview

This study is a multicenter, randomized, controlled, open-label trial. A total of 150 patients with COPD, non-CF bronchiectasis or asthma, and *P. aeruginosa*-positive lower respiratory tract samples, will be randomly assigned with a 1:1 ratio to either no antibiotic treatment (control group) or anti-pseudomonal antibiotic treatment with intravenous beta-lactam and oral ciprofloxacin (intervention group) for 14 days.

The monitoring guideline outlined below will adhere to the protocol approved by the Ethics Committees of all participating sides (H-15010949), Danish Medicines Agency (EudraCT:2015-003399-58) and the Danish Data Protection Agency.

Data Quality and Safety Review Plan and Monitoring

A. Subject Recruitment

Review of the rate of subject accrual and compliance with inclusion/exclusion criteria will occur regularly to ensure that a sufficient number of participants are being enrolled and meet eligibility criteria. Recruitment rate will be reported by a recruitment graph.

B. Justification of Sample Size

We expect an effect size of 20% absolute reduction (30% relative reduction) of exacerbation or mortality in the antibiotic group:

- Sixty-seven percent of study participants in the antibiotic-free group have exacerbated or died within 12 months
- Forty-seven percent of study participants in the antibiotic group have exacerbated or died within 12 months

The power to avoid type II error is 80% (1-β) at a two-sided 5% significance level. We used a group-sequential design, allowing for one interim analysis at half target recruitment. Based on these estimates and indicative figures, a total of 150 patients should be included (i.e., 75 patients in each group).

C. Stopping Rules

This study will be stopped prior to its completion if the intervention is associated with adverse effects that call into question the safety of the intervention or failure of feasibility.

D. Designation of an Independent Monitor

The Independent Monitor for this study is the GCP (Good Clinical Practice)-unit at Copenhagen University Hospital.

E. Safety Review

The DSMB review will focus:

i) Baseline characteristics

ii) Adherence data

iii) Follow-up (missing data)

iv) Outcome data

v) Accrual data (data for feasibility for completion)

We have planned the interim analysis when all the data from the first 75 patients have been entered into the database (half of the patients recruited). The DSMB may, apart from this planned interim analysis, decide to request an extra-ordinary interim analysis at any time point. This will be blinded to the investigators.

Analyses

The analyses described in this document will be performed in a blinded manner ("group A" and "group B") by a trial statistician with guidance from the sponsor, once the data have been entered, cleaned and released for use. This document provides a description of the statistical analyses that will be performed for the evaluation of the primary and secondary endpoints of the TARGET ABC trial. The analyses described in this document are compatible with the recommendations of the CONSORT 2010 statement.

A. Analysis population

Data will be analyzed using intention-to-treat (ITT) principles. All randomized patients will be analyzed in the groups to which they were originally allocated to, regardless of whether they actually received the intended treatment or whether a protocol violation or deviation occurred.
Patients who withdrew consent for use of their data will not be included in any analysis. Only the facts that they were enrolled into the trial, including the study group to which they were allocated, and that they withdrew consent, will be reported. Patients who withdraw consent to the intervention, but allowed for data analysis, will be included in the ITT as well as a modified ITT analysis.
Two-sided 5% significance levels will be used to identify statistically significant results. All confidence intervals reported will be 95% confidence intervals. All analyses will be performed using SAS software version 9.4.

B. Definitions

Baseline: day 1 (randomisation)
Follow-up visits: 14, 30, 60, 90 and 365 days.

C. Baseline characteristics

The following baseline characteristics of the study population will be summarized separately within each randomized group:

- Age, median (interquartile range)
- Male, n (%)
- Body mass index, median (interquartile range)
- Smoking status: current smoker, ex-smoker, never-smoker, n (%)
- Pack-years history, median (interquartile range)
- FEV1 % predicted, median (interquartile range)
- Use of long-term oxygen treatment (LTOT), n (%)
- Home-NIV, n (%)

For each variable, the percent of missing values will be reported.

D. Adherence data

Adherence to the antibiotic intervention will be evaluated by computer registered information on administered antibiotics and medicine log filled by the participants. Adherence will be reported as good (> 60%), partial (40-60%), and non-adherent (< 40%).

E. Follow-up (missing data)

The proportion of patients followed for each outcome data parameter will be reported for the predefined primary and secondary outcomes. Possible reasons for missing outcome data will be reported.

F. Outcome data (for the pre-planned interim analysis)

The interim analysis will be performed, when we have included 50% of the sample size (75 patients)

- Primary outcome:

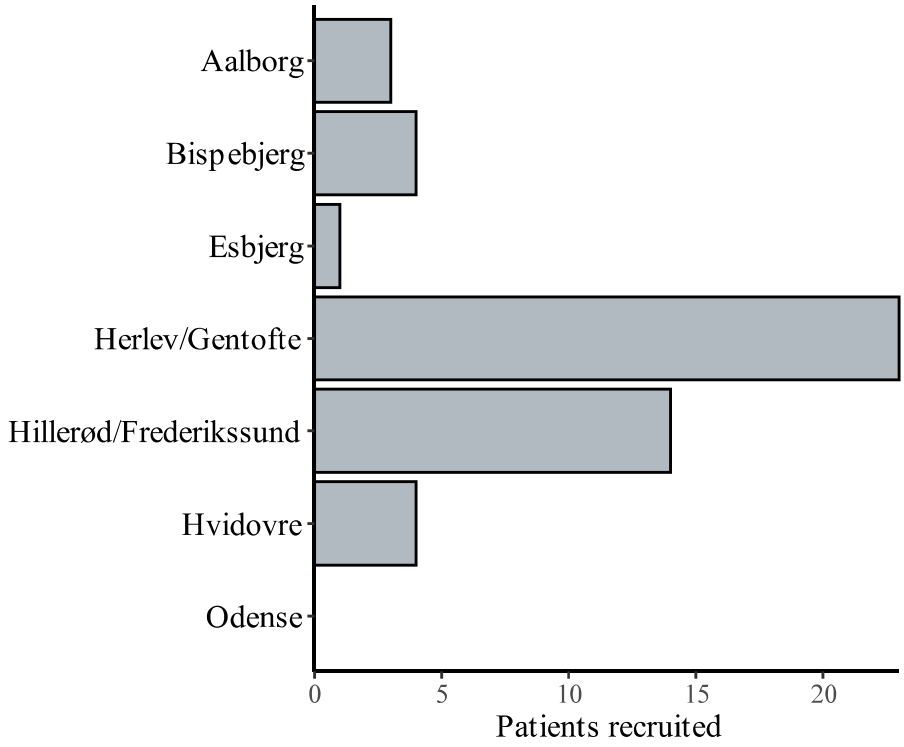
Co-primary outcome: i) Time to prednisolone and/or antibiotic requiring exacerbation or death, in primary or secondary health care sector, from day 20 to day 365 from randomization ii) Days alive and without hospitalisation from day 20 to day 365 from randomisation ("Days alive and without hospitalisation from day 20 to day 365 from randomisation" was degraded to the first secondary outcome by the trial statistician in agreement with the coordination investigator and study director since this outcome would be severely underpowered because of the premature closure of the trial. This decision was taken before the database was unblinded to the analysis (see Supplementary page 24).

- Secondary outcome: All-cause mortality at 365 days
- Secondary outcome: Microbiological cure at 90 days
- Explaining data

G. Accrual data (data for feasibility for completion)

Recruitment rate will be compared to the planned recruitment in order to assess the futility of the trial.
Data will be presented as the total number of recruited patients per site.

PATIENT RECRUITMENT PER TRIAL SITE



DSMB ENDORSMENT LETTER FOR EARLY TERMINATION

01MAR2023

Attention
COP:TRIN Steering Committee and Trial Leadership for the TARGET-ABC trial
Principal Investigator: Prof. Jens-Ulrik Stæhr Jensen

The trial DSMB thank the trial leadership for data regarding the accrual rate in the TARGET-ABC trial.

These data were reviewed without further interim analysis data, since the trial has not reached the recruitment of 50% of the target sample size yet, and thus, no ordinary interim analysis has been performed.
We, as DSMB, have not seen any reason to demand an extra ordinary full interim analysis, and we still do not consider this a reasonable step to take at the current point either.

The trial steering committee has decided to take motion to terminate the trial prematurely, since the empiric feasibility to complete the trial, does not seem present.

The DSMB has considered this. We make the following comments:

In our opinion this is a very well planned and executed trial and the study team did tremendous efforts trying to increase recruitment. Congratulations for this!

However, we agree with the conclusion of the steering committee that at this point stopping the trial with publication of results is inevitable. Although the trial will be underpowered at this point, we may still learn something from the results, and later integration in a meta-analysis would be possible to increase sample size.

Sincerely,

Tobias Wirenfeldt Klausen, Trial statistician, Copenhagen Tobias Wirenfeldt Klausen date 20230302

Prof. Tor Biering Sørensen, Copenhagen Tor Biering-Sørensen date 7/3-23

Prof. Philipp Schuetz, Basel date 9/3/23

NOTE TO STATISTICAL ANALYSIS PLAN PRIOR TO DATABASE UNLOCKING

Herlev og Gentofte Hospital

Medicinsk afdeling C

Telefon 28938168
Direkte 38673057

Note for SAP before database unlock

Journal-nr.:
TARGET_ABC_note_analysis
Ref.: JUSJ

Dato: 15. May 2023

Participants:

Analysis team:

Mr. Tobias Wirenfeldt Klausen, MSc, Biostatistician

Mr. Alexander Svorre Jordan, BSc, analysis assistant

Study Director: Professor Jens-Ulrik Stæhr Jensen, MD, PhD

National Trial Coordinator: Mrs. Josefin Viktoria Eklöf, MD, PhD

Subject:

Analysis of the Primary Outcome in the TARGET-ABC trial

The above persons met the 15th May 2023 to discuss the final analysis before database unlock.

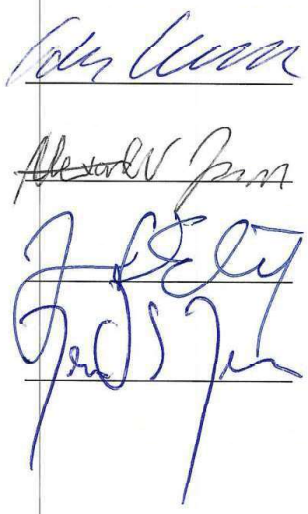
DECISION:

The analysis team and the representatives from the steering committee (study director and national coordinators) unanimously decided to analyze the primary outcome as a "time to event" analysis. The "Days Alive And Out of Hospital" (DAOH) analysis was thus degraded to the first secondary outcome.

Reason: Recruitment was stopped at approx.. 1/3 of planned and the team was concerned that the initial power analysis for the DAOH would not give sense with the considerably reduced power.

Opposite, it was decided that the more conventional and well-known analysis using the time-to-event analysis would be easier to conclude on. Specifically, the team was concerned that power issues would lead to discrepancies between the two ways of considering the primary analysis.

Tobias Wirenfeldt Klausen, MSc	15 May 2023
Alexander Svorre Jordan, BSc	15 May 2023
Josefin Viktoria Eklöf, MD, PhD	15 May 2023
Jens-Ulrik Stæhr Jensen, MD, PhD	15 May 2023



EN DEL AF
GREATER
CoPENHAGEN

SUPPLEMENTAL TABLES AND FIGURES

Table 1: Cox regression for primary endpoint, exacerbation or death within days 20 to 365 in the ITT population for selected patient subgroups.

		Antibiotic group	Control group		
		N events (%)	N events (%)	Crude HR (95% CI)	P value
	Patients with COPD (n=40)	13 (68)	20 (95)	0·46 (0·23—0.93)	0·030
	Patients with asthma (n=11)	5 (63)	3 (100)	0·75 (0.18—3·19)	0·70
	Patients with bronchiectasis (n=20)	7 (58)	8 (100)	0·37 (0·13—1·03)	0·056

Table 2. Number of patients with *Pseudomonas aeruginosa*-positive sputum samples during the planned study visits.

	Day 14	Day 30	Day 60	Day 90	Day 360
Study group					
Antibiotic group	1	8	9	12	5
Control group	15	11	8	12	6
Total in both study groups	16	19	17	24	11

Table 3: Post-hoc analyses for comparing changes in selected secondary outcome measures at other time points.

	Antibiotic group (n = 26)	Control group (n = 23)	Difference (95% CI)	P value	Number missing
Change in CAT from baseline to day 30 day, mean (95% CI)	-4·0 (-6·8—-1·1)	-2·5 (-4·8—-0·1)	-2·2 (-5·7—1·3)	0·21	7
Change in CAT from baseline to day 365, mean (95% CI)	-1·8 (-5·0—1·4)	-1·9 (-5·7—1·8)	-0·9 (-5·4—3·6)	0·69	12
Change in FEV1 (L) from baseline to day 30 day, mean (95% CI)	0·08 (0·01—0·15)	-0·03 (-0·08—0·02)	0·11 (0·03—0·19)	0·011	7
Change in FEV1 (L) from baseline to day 365, mean (95% CI)	0·06 (-0·04—0·16)	-0·03 (-0·10—0·04)	0·09 (-0·03—0·21)	0·14	18

CAT=COPD assessment test. FEV_1=forced expiratory volume the first second.

Table 4: Adverse events in the study population

	Antibiotic group (n = 26)	Control group (n = 23)	P value
Severe adverse events			
Infections and parasitic diseases, n (%)	1 (4)	0 (0)	
Metabolism and nutrition, n (%)	0 (0)	1 (4)	
Airways, thorax and mediastinum, n (%)	1 (4)	1 (4)	
Kidneys and urinary tract, n (%)	1 (4)	0 (0)	
Any severe adverse event, n (%)	3 (12)	1 (4)	0·61
Non-severe adverse events			
Blood and lymphatic system, n (%)	0 (0)	4 (17)	
Immune system, n (%)	1 (4)	0 (0)	
Heart, n (%)	2 (8)	0 (0)	
Airways, thorax and mediastinum, n (%)	0 (0)	1 (4)	
Gastrointestinal tract, n (%)	1 (4)	0 (0)	
Skin and subcutaneous tissues, n (%)	1 (4)	0 (0)	
Bones, joints, muscles, and connective tissues, n (%)	1 (4)	1 (4)	
Kidneys and urinary tract, n (%)	1 (4)	1 (4)	
Any non-severe adverse event, n (%)	6 (23)	5 (22)	1·00
Any adverse event, n (%)	9 (34)	6 (26)	0·55

Figure 3: Days alive and out of hospital from day 20 to 365

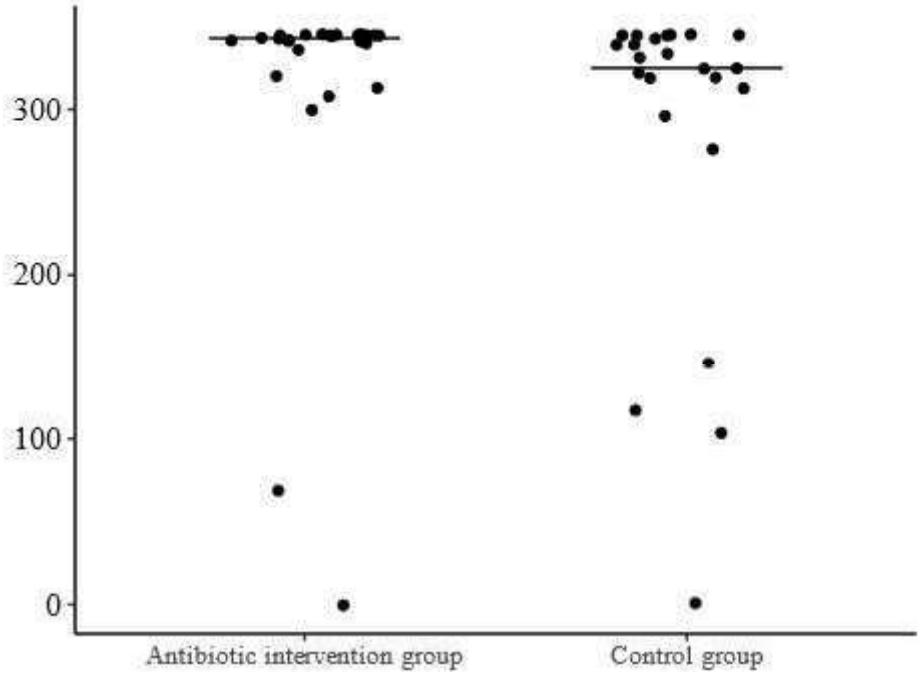


Figure 4. Mortality within 365 days

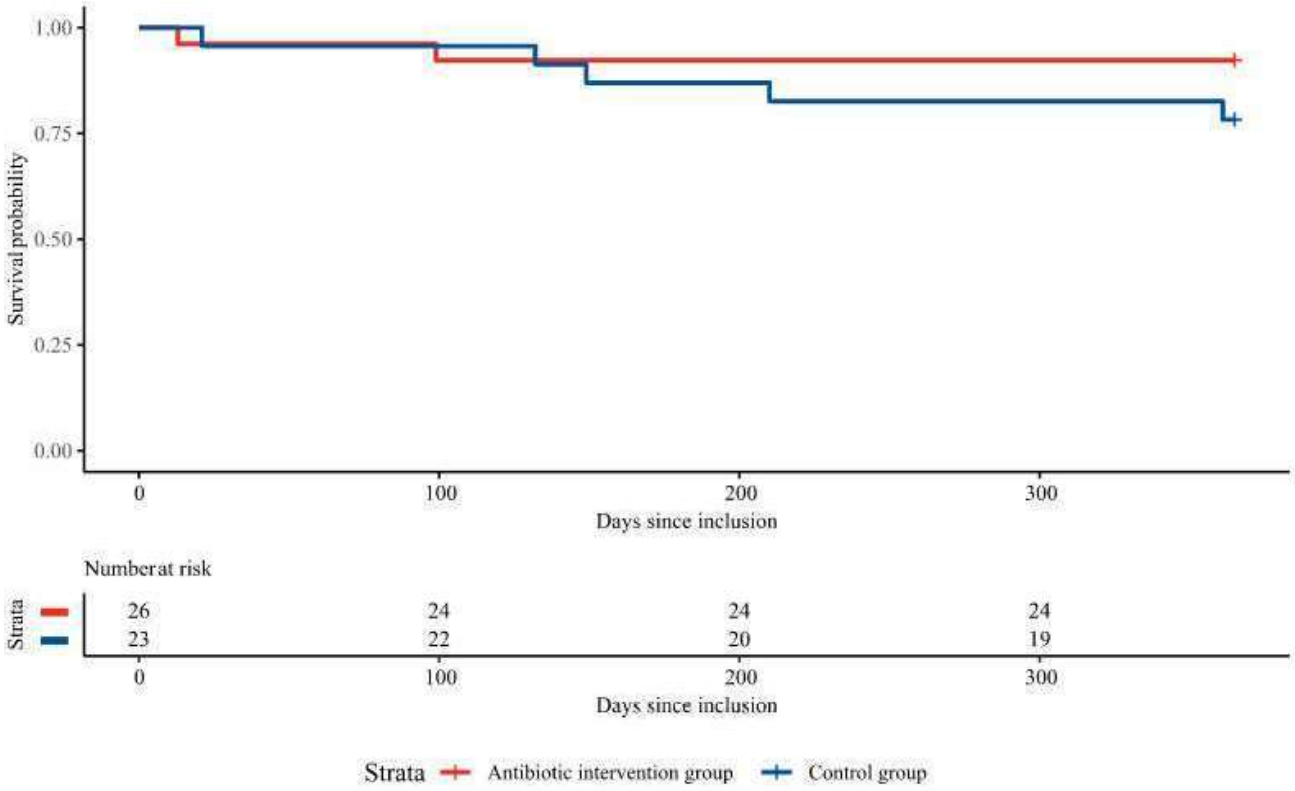


Figure 5. Number of hospital admissions with exacerbation within 365 days from randomization

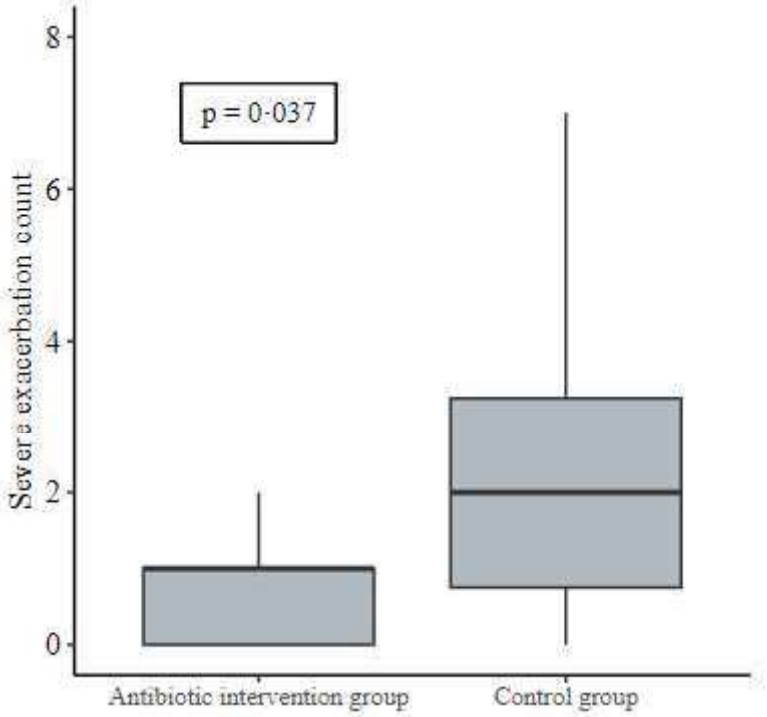


Figure 6. Changes in CAT scores (A), body mass index (B), and FEV_1 (C) from randomization to day 365