Inhaled, dual-release liposomal ciprofloxacin in non-cystic fibrosis bronchiectasis (ORBIT-2) – a randomised, double-blind, placebo-controlled trial

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Online Data Supplement

Methods

Inclusion Criteria

Patients must have met all of the following criteria for inclusion in the study:

1. Were willing and able to provide written informed consent.
2. Were males or females 18 to 80 years of age, inclusive, who were able to walk.
3. Had a confirmed diagnosis of non-CF bronchiectasis per computerized tomography (CT).
4. Had a confirmed history of at least two pulmonary exacerbations treated with a course of antibiotics within the last 12 months.
5. Had been off any anti-pseudomonal antibiotic for a minimum of 28 days prior to Visit 1.
6. Had a forced expiratory volume in 1 second (FEV_1) of more than 25% of predicted values at the Screening Visit (Visit 0).
7. Had positive documented *P. aeruginosa* in a sputum/deep-throat cough swab culture (or bronchoalveolar lavage [BAL]) within 6 months prior to the Screening Visit (Visit 0) and in the sputum/deep-throat cough swab culture collected at the Screening Visit (Visit 0).
8. Were clinically stable and capable of performing the 6-minute walk test without supplemental oxygen in the opinion of the Investigator.
9. Were willing to comply with the requirements for participation in the study.
10. Were willing to use an acceptable method of contraception during the study.
11. Female patients of childbearing potential must have provided a negative pregnancy test result at the Screening Visit and must have been using an acceptable method of contraception for 3 weeks prior to the first dose of study drugs and for 30 days after the last dose of study drugs. Acceptable methods of contraception for women were orally administered hormonal contraceptives, surgical intervention, intrauterine device (IUD), and sexual abstinence. If a hormonal contraceptive was utilized as a method of contraception, the same method must have been used for at least 3 months prior to Visit 1
12. To be considered "not of childbearing potential", female patients must have been at least 2 years postmenopausal, or have been irreversibly surgically sterilized by hysterectomy, oophorectomy, or bilateral tubal ligation for at least 3 months prior to the first dose of study drugs.
13. Male patients whose female partners were of childbearing potential (definition as above) must have agreed to use an acceptable method of contraception (as listed above) for the duration of the study treatment and for 30 days after the last dose of study drugs.

Exclusion Criteria

Patients who met any of the following exclusion criteria were not included in the study:

1. Had a known local or systemic hypersensitivity to fluoroquinolone or quinolone antibiotics.
2. Had a pulmonary exacerbation during the Screening Phase as defined as requiring treatment with inhaled, oral, or IV antibiotics prior to the first dose of study drugs.
3. Had a diagnosis of CF.
4. Had a diagnosis of allergic bronchopulmonary aspergillosis.
5. Had received any IV, oral, or inhaled anti-pseudomonal antibiotic within 28 days prior to Visit 1.
6. Had used tizanidine within 28 days prior to Visit 1.
7. Had initiated supplemental oxygen within 28 days prior to Visit 1.
8. Had used any intravenous or intramuscular corticosteroid or had used oral corticosteroid >10 mg/day or >20 mg every other day within 28 days of Visit 1.
9. Had changes in either the treatment regimen or initiation of treatment with any of the following medications within 28 days prior to Visit 1:

    - Azithromycin,
    - Hypertonic saline,
    - Mucolytics,
    - Bronchodilator medications,
    - Oral corticosteroid.

10. Had changes in physiotherapy technique or schedule within 28 days prior to Visit 1.

11. Had a history of solid organ (e.g., lung) transplantation.
12. Had a history of non-tuberculosis mycobacteria requiring treatment within 12 months prior to Visit 1.
13. Had serum creatinine levels ≥1.5x upper limit of normal (ULN) at the Screening Visit (Visit 0).
14. Had serum transaminase levels >3x ULN at the Screening Visit (Visit 0).
15. Had a febrile illness within 1 week prior to Visit 1.
16. Had massive hemoptysis (greater than or equal to 300 mL or requiring blood transfusion) within 6 months prior to Visit 1.
17. Had used any over-the-counter product, herbal product, diet aid, hormone supplement, etc., within 7 days prior to dosing unless approved by both the Investigator and the Sponsor.
18. Had received an investigational drug or device within 28 days prior to Visit 1.
19. Had any serious or active medical or psychiatric illness, which in the opinion of the Investigator, would have interfered with the patient's treatment assessment, or compliance with the protocol.
20. Had a history or suspicion of unreliability, poor cooperation, or non-compliance with medical treatment.
21. Were unable to use nebulizers.
22. Were unable either to understand the instruction for use of the study drugs or to complete the QoL questionnaire at Visit 1.
23. Had previously enrolled in this study.
24. Were pregnant, planned to become pregnant during the study, were nursing mothers or were unwilling to use an acceptable method of contraception for the duration of the study.

Patients who met all inclusion and none of the exclusion criteria and met the following sputum criteria following screening assessments were enrolled in the study:

- Sputum positive for *P. aeruginosa* in the screening sputum sample, and
- At least one strain of *P. aeruginosa* sensitive to ciprofloxacin (defined as MIC ≤ 1μg/mL).

Up to 2 additional sputum samples could be submitted per subject during the 14 day screening period if ciprofloxacin-sensitive *P. aeruginosa* was not identified in the initial sputum sample. Hence, subjects who cultured any ciprofloxacin-sensitive *P.aeruginosa strain* in the screening sample/s were eligible, even if all other strains were ciprofloxacin- resistant.

Treatments administered and blinding

Central randomization was used in this study to protect the planned balanced 1:1 active to placebo ratio. A balanced randomization method was used to place equal numbers of patients treated with active study drug and placebo. Randomized patients were stratified into two groups of reported annual pulmonary exacerbations, namely a group reporting 2 or 3 annual pulmonary exacerbations, and the other group reporting 4 or more pulmonary exacerbations per year. No study center personnel involved in the day-to-day clinical conduct of the study had access to the code (the unblinded pharmacist had access to the code).

DRCFI (6 mls total) consisted of 3 mLs of liposomal ciprofloxacin for inhalation (CFI) 50 mgs/ mL and 3 mLs of free ciprofloxacin for inhalation (FCI) 20 mgs/mL (both manufactured by Enzon Pharmaceuticals), each provided to subjects in separate vials. Matched placebo consisted of 3 mLs of control liposomes for inhalation (Enzon Pharmaceuticals) and 3 mLs of normal saline, provided in separate vials. Subjects were required to open one vial of each of the 2 components of their supplied study drug into the nebulizer, a PARI LC Sprint nebuliser powered by a PARI Turbo Boy-S compressor (PARI Respiratory Equipment, Richmond, VA, USA) prior to administration.

All formulations were packaged in single-use 5-mL vials that contained 3 mL of solution.

DRCFI (ARD-3150) consisted of the following:

- *Ciprofloxacin for Inhalation (CFI)*, 50 mg/mL (manufactured by Enzon Pharmaceuticals) contained liposomally encapsulated ciprofloxacin (150 mg of ciprofloxacin expressed as ciprofloxacin hydrochloride in 3 mL of aqueous liposomal dispersion containing high purity [HP] cholesterol, HSPC, ammonium sulfate, histidine, sodium chloride, and water for injection).

- *Free Ciprofloxacin for Inhalation (FCI)*, 20 mg/mL (manufactured by Enzon Pharmaceuticals) contained ciprofloxacin hydrochloride (60 mg in 3 mL), sodium acetate, glacial acetic acid, and water for injection.

Placebo for Inhalation consisted of the following:

- *Control Liposomes for Inhalation (CLI)*, 5 mg/mL lipids (manufactured by Enzon Pharmaceuticals) contained HP cholesterol, HSPC, ammonium sulfate, histidine, sodium chloride, and water for injection in 3 mL.
- *Normal Saline* (manufactured by Baxter) contained 0.9% sodium chloride and water for injection in 3 mL.

This study was performed in a double-blind manner. The study drugs were supplied in identical 5-mL vials. The CFI formulation was similar in appearance to the CLI formulation lid concentration, and the FCI formulation was similar in appearance to the normal saline.

The study blind was not to be broken except in a medical emergency (where knowledge of the study drugs received would not affect the treatment of the emergency) or regulatory requirement.

Outcome measures

The parameter used for the primary efficacy analysis was bacterial load where bacterial load was defined as *P. aeruginosa* density in sputum (log_{10}) CFU/gram of sputum. The primary variable of analysis was the mean change in *P. aeruginosa* load from Baseline to Day 28, where Baseline was the mean of *P. aeruginosa* load at Screening (Visit 0) and Day 1 (Visit 1) and the endpoint value was the Day 28 assessment.

Additional (secondary) efficacy variables included: Relative change in *P. aeruginosa* load from Baseline to Day 28; Microbiological efficacy; Time to first pulmonary exacerbation (defined as the time in days from first dose to first occurrence of a clinically defined pulmonary exacerbation event and were calculated as first dose date to onset date of first pulmonary exacerbation + 1); Number of pulmonary exacerbations; Severity of pulmonary exacerbations; Length of time to resolve pulmonary exacerbations (time was calculated as onset date to resolution date + 1); Changes and relative changes in spirometry; Changes in QoL; changes in 6mwt; Isolation of pathogens other than *P. aeruginosa* from sputum and changes in the ciprofloxacin minimum inhibitory concentration (MIC) for *P. aeruginosa* from sputum.

Assessment of outcome measures

Sputum samples were collected and transferred by courier from the trial sites to the central processing laboratory on the same day, stored with a refrigerated gel pack. Samples were processed immediately upon receipt. Samples were graded for colour (according to 'Bronkotest') and recorded as mucoid/ mucoid-mucopurulent/ mucopurulent/ mucopurulent-purulent or purulent. Samples were split into aliquots to allow pathogen isolation/identification, ciprofloxacin sensitivity testing of organisms and quantitative bacteriology and then homogenized 1:1 with Sputasol (Oxoid Ltd, Basingstoke, UK). Undiluted sample was inoculated onto horse blood, chocolate and *Pseudomonas* (CFC) agar plates and incubated aerobically (5% CO_2 for chocolate agar) at 35°C. For quantitative bacterial counts, serial dilutions of neat sputum from 1:10 to 1:100000 were prepared with sterile 0.9% saline and 10 µL samples from each dilution inoculated onto chocolate agar and incubated aerobically. Viable numbers of potential pathogenic organisms were read at 24 hours and the count from the lowest dilution plate that contained between 30 and 300 discrete colonies of each organism was recorded. Viable bacteria numbers were recorded as colony-forming units (CFU) per mL of original sputum volume. Bacterial identification was confirmed using: API20NE (*P.aeruginosa* and non-fermentive gram negative bacilli), optochin sensitivity (*Streptococcus pneumoniae*), XV factor +ve (*Haemophilus influenza*), Tributyrin/ Oxidase/ dnase +ve (*Moraxhella catarrhalis*), latex/ dnase +ve (Staphylococcus aureus) and Vitek2 GN card (coliforms). Ciprofloxacin sensitivity testing was performed for all isolated bacterial pathogens by Etest (AB Biodisks, Solna, Sweden) MIC on Mueller-Hinton agar plates. Ciprofloxacin sensitivity was determined according to CLSI systemic breakpoints (eg for *P.aeruginosa* MIC ≤ 1 mg/mL).[1]

Pulmonary Exacerbation was defined as abnormalities in four of the following nine symptoms, signs, or laboratory findings[2]: 1. Change in sputum production (consistency, color, volume, or hemoptysis); 2. Increased dyspnea (chest congestion or shortness of breath); 3. Increased cough; 4. Fever (>38°C); 5. Increased wheezing; 6. Decreased exercise tolerance, or increased malaise, fatigue, or lethargy; 7. FEV_1 or FVC decreased 10% from a previously recorded value; 8. Radiographic changes indicative of a new pulmonary process; and 9. changes in chest sounds.

Pulmonary exacerbations were assessed from Day 1 to Day 168 using the above criteria and the following was recorded for each patient: Date of onset and resolution of each pulmonary exacerbation; Radiographic confirmed infective processes (lung infections); Treatment for each pulmonary exacerbation including requirement of hospitalization in relationship to the pulmonary exacerbation, adjustments in treatment, including increase in frequency of current therapy in relationship to the pulmonary exacerbation; use of any antibiotic; and use of parenteral antibiotics.

Spirometry was assessed for the following: FEV_1 (liters); FEV_1 % predicted; FVC, and FVC % predicted. FEV_1/FVC ratio, Peak Expiratory Flow (PEF_{25-75}), and Peak Expiratory Flow Rate (PEFR; also known as Forced Expiratory Flow Rate [FEF_{25-75}]) were also recorded for quality assurance review, but not assessed.

Spirometry equipment was monitored for calibration drift to standardize measurement across sites by Respiratory Quality Assurance. Additionally, Respiratory Quality Assurance provided training and a quality review of all measurement per the American Thoracic Society guidelines.[3]

At visit 1, spirometry was performed before and 60 minutes after inhalation of study drug, without bronchodilator premedication.

Safety Variables

Safety was monitored in this study by collection of AEs, clinical laboratory measures, and vital signs. It was noted that all untoward events or experiences were reported as AEs regardless of whether they were identified by clinical observation, patient reporting, physical examination, clinical laboratory test results, electrocardiography, or any other examination or test.

Data analysis

Kaplan-Meier Survival Curves were presented for time to patient's drop out defined as time to when the patient was withdrawn from study treatment. If a patient was lost from the population prior to study termination withdrawal that was considered as the event of interest, the patient was censored.

Summaries of continuous variables included number of patients, mean, median, minimum, maximum, and standard deviation (SD). Summaries of categorical variables included numbers of patients in each category. The variables to be summarized included: Age, gender, and race; weight and height; and baseline pulse rate, systolic blood pressure, diastolic blood pressure, and pulse.

The primary analysis was done on the Full Analysis Set (FAS; all subjects who received at least one dose of study drug, hereafter referred as the Modified Intention to Treat, mITT, group) using analysis of covariance (ANCOVA) main effects parametric model with effects for the randomization stratification as a blocking variable, the baseline value for bacterial load as a covariate, and the treatment effect. The hypothesis to be tested was that mean change from Baseline to Day 28 of *P. aeruginosa* density $\log_{10}$ CFU/gram in sputum culture for the group treated with ARD-3150 was equal to that of the group treated with placebo. As supporting analysis, relative change from Baseline was also summarized. In the case of missing data, last observation carried forward (LOCF) was used under the assumption that the LOCF analysis was conservative and biased toward equivalence.

Additional efficacy variables included microbiological efficacy, time to first pulmonary exacerbation, number of pulmonary exacerbations, severity of pulmonary exacerbations, length of time to resolve pulmonary exacerbations, changes and relative changes in spirometry, 6-minute walk test, and changes in QoL. In addition to direct SGRQ scores, derived SGRQ scores per SGRQ manual were considered. The derivation was based on the SGRQ Manual v2.2.[4] As supporting analyses, relative change from Baseline to sputum *P. aeruginosa*

load, spirometry (FEV_1 and FVC) and 6-minute walk were also summarized. Analyses of *P. aeruginosa* load, FEV_1 and FVC were performed with and without LOCF for each applicable time point. These secondary variables were summarized descriptively by treatment group if data were available. P-values from relevant statistical tests presented acted as supplementary information.

Kaplan Meier Survival Curves were created for time to first pulmonary exacerbation by treatment group. If a patient was lost from the population prior to first pulmonary exacerbation occurred that was considered as the event of interest, the patient was censored. A graph was also added that showed the time to first pulmonary exacerbation, regardless of any other factors. This was part of the Kaplan-Meier Survival Analysis.

Other efficacy analyses may also have been added if data were available that included summary of initiation of antibiotics for pulmonary exacerbations or other infectious indications (e.g., sinus infections), correlation analysis between minimum inhibitory concentration (sensitive, intermittent or resistant strains) and responders (patients who experienced pulmonary exacerbations), correlation analysis between mucopurulent and purulent sputum.

Based on Study ARD-3150-0703, a conservative estimate of the mean difference between CFI and placebo was 4 $\log_{10}$ CFU/gram in sputum culture and a conservative estimate of the standard deviation (SD) of the change from Baseline was 3.5 $\log_{10}$ CFU/gram in sputum culture. A sample size of 40 patients randomized in a balanced ratio of 1:1 provided more than 90% power to detect a difference of 4 $\log_{10}$ CFU/gram in sputum culture in mean change from Baseline to Day 28 based on a 2-sided, 2-sample t-test with $\alpha = 0.05$ and assuming a common SD of 3.5 $\log_{10}$ CFU/gram in sputum culture.

References

1. Clinical and laboratory standards institute. Performance standards for antimicrobial disk susceptibility tests; approved standard – tenth edition. M100-S19 2009; 29 (3).

2. Fuchs HJ, Borowitz DS, Christiansen DH, *et al*. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. *N Engl J Med* 1994;**331**:637-42.

3. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Interpretative strategies for lung function tests. *Eur Respir J* 2005; 26: 948–968.

4. Jones PW, Forde Y. The St. George's Respiratory Questionnaire Manual v2.2. Respiratory Medicine St. George's, University London; 2008.

Table 1. Additional secondary outcome measures not reported in the main paper.

	Placebo	DRCFI	P value
	Change from baseline to day 28		
Relative change in sputum *P.aeruginosa* load (%)	+5 (-100, +346)	-100 (-100, +60)	0.004
Severity of pulmonary exacerbations (n)			
- mild	1	2	0.49
- Moderate	14	7	
- severe	2	2	
Days to exacerbation resolution	22.3 (7,62)	20.3 (11, 63)	0.82

(Results are median (range) except where otherwise indicated. DRCFI – dual release ciprofloxacin for inhalation)

Figure Legends

Figure 1: Overall Study Design and Plan ORBIT-2

(DRCFI – dual release ciprofloxacin for inhalation; CFI – liposomal ciprofloxacin for inhalation; FCI – free ciprofloxacin for inhalation; ON – represents 28 day periods during which subjects inhaled trial medication once daily; OFF – represents 28 day periods during which subjects did not inhaled trial medication)

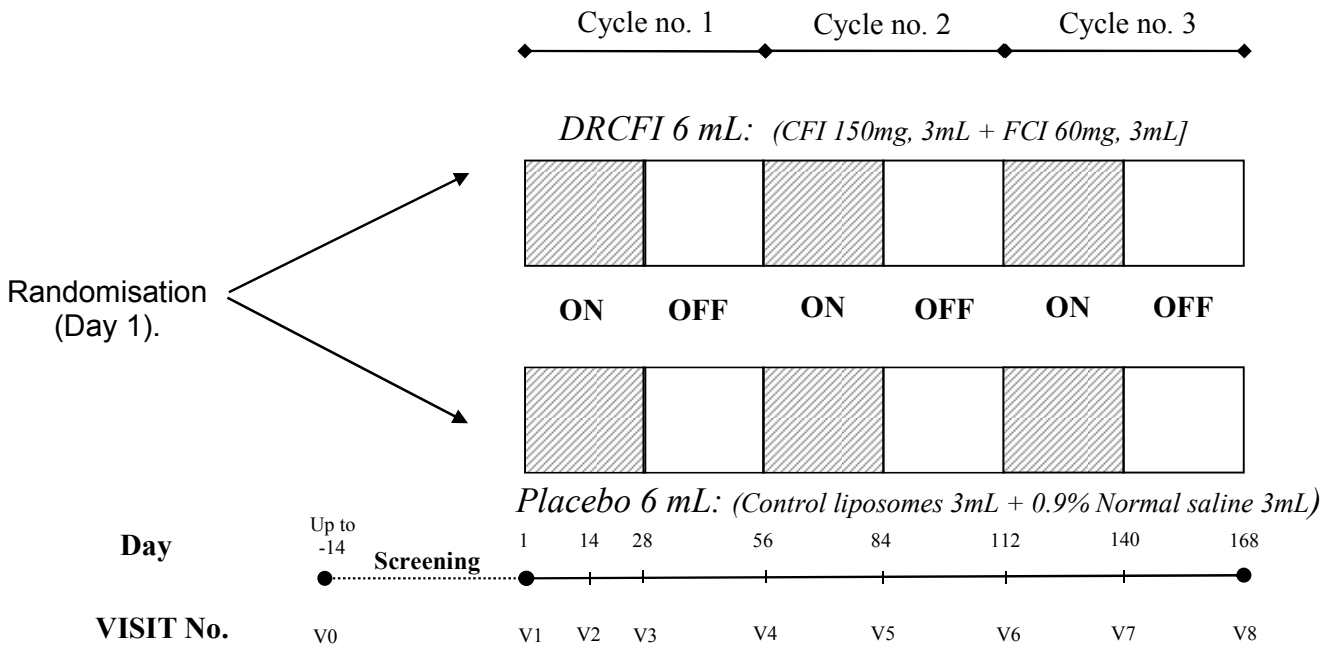
Cycle no. 1
Cycle no. 2
Cycle no. 3
DRCFI 6 mL: (CFI 150mg, 3mL + FCI 60mg, 3mL]
Randomisation
(Day 1).
ON
OFF
ON
OFF
ON
OFF
Placebo 6 mL: (Control liposomes 3mL + 0.9% Normal saline 3mL)
Day
Up to
-14
Screening
1
14
28
56
84
112
140
168
VISIT No.
V0
V1
V2
V3
V4
V5
V6
V7
V8

Figure 1.