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ORIGINAL ARTICLE

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

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ABSTRACT

Background The delivery of antipseudomonal antibiotics by inhalation to *Pseudomonas aeruginosa*-infected subjects with non-cystic fibrosis (CF) bronchiectasis is a logical extension of treatment strategies successfully developed in CF bronchiectasis. Dual release ciprofloxacin for inhalation (DRCFI) contains liposomal ciprofloxacin, formulated to optimise airway antibiotic delivery.

Methods Phase II, 24-week Australian/New Zealand multicentre, randomised, double-blind, placebo-controlled trial in 42 adult bronchiectasis subjects with ≥2 pulmonary exacerbations in the prior 12 months and ciprofloxacin-sensitive *P aeruginosa* at screening. Subjects received DRCFI or placebo in three treatment cycles of 28 days on/28 days off. The primary outcome was change in sputum *P aeruginosa* bacterial density to the end of treatment cycle 1 (day 28), analysed by modified intention to treat (mITT). Key secondary outcomes included safety and time to first pulmonary exacerbation—after reaching the pulmonary exacerbation endpoint subjects discontinued study drug although remained in the study.

Results DRCFI resulted in a mean (SD) 4.2 (3.7) $\log_{10}$ CFU/g reduction in *P aeruginosa* bacterial density at day 28 (vs −0.08 (3.8) with placebo, p=0.002). DRCFI treatment delayed time to first pulmonary exacerbation (median 134 vs 58 days, p=0.057 mITT, p=0.046 per protocol). DRCFI was well tolerated with a similar incidence of systemic adverse events to the placebo group, but fewer pulmonary adverse events.

Conclusions Once-daily inhaled DRCFI demonstrated potent antipseudomonal microbiological efficacy in adults with non-CF bronchiectasis and ciprofloxacin-sensitive *P aeruginosa*. In this modest-sized phase II study, DRCFI was also well tolerated and delayed time to first pulmonary exacerbation in the per protocol population.

Key messages

What is the key question?

- Does the once-daily inhalation of a dual release liposomal ciprofloxacin formulation reduce airway bacterial load in non-cystic fibrosis bronchiectasis subjects with at least one ciprofloxacin-sensitive *Pseudomonas aeruginosa* bacterial strain, without tolerability issues?

What is the bottom line?

- Dual release ciprofloxacin for inhalation appeared well tolerated and resulted in large reductions in airway *P aeruginosa* bacterial load.

Why read on?

- In the per protocol population, dual release ciprofloxacin for inhalation also delayed time to first pulmonary exacerbation, a key secondary outcome measure.

INTRODUCTION

Non-cystic fibrosis (CF) bronchiectasis remains a condition for which there are few therapies of proven benefit and no licensed therapies to date. The need for well-designed randomised-controlled trials to inform clinical management is a priority. Pulmonary *Pseudomonas aeruginosa* infection in non-CF bronchiectasis is associated with worse quality of life,[1] more pulmonary exacerbations[2] and more rapid lung function decline.[3] The direct delivery of antipseudomonal antibiotics to the respiratory tract of these subjects by inhalation represents a logical treatment paradigm and the proven efficacy of this approach in CF[4] provides an appealing blueprint. However, prior attempts to translate proven CF therapies into non-CF bronchiectasis have been unsuccessful to date. Neither recombinant human DNase I nor tobramycin solution for inhalation (TSI/ TOBI) improves clinical outcomes in this patient population.[5 6]

In spite of microbiological efficacy in non-CF bronchiectasis, inhaled TOBI increases respiratory adverse events (AEs).[6] This uncoupling of microbiological and clinical efficacy has been reproduced in another randomised-controlled study of TOBI[7] and poor tolerability confirmed in other studies of both TOBI[8] and alternative tobramycin solutions.[9] The poor tolerability of inhaled aminoglycoside antibiotics in non-CF bronchiectasis may relate to high concentrations of antibiotic contacting the airways during inhalation and the better outcomes reported with low dose nebulised gentamicin in a recent study seem to support this.[10]

Liposomal encapsulation of inhaled antibiotics may improve tolerability without sacrificing

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microbiological efficacy by minimising the amount of free antibiotic in direct contact with the airway during inhalation and yet still releasing adequate drug dose to the lower airways. Dual release ciprofloxacin for inhalation (DRCFI, Pulmaquin; Aradigm Corporation, Hayward, California, USA) is a mixture of liposomal and free ciprofloxacin. This formulation has been developed with a view to optimising airway delivery characteristics, being distinguished by both an immediate effective dose (free component) and sustained delivery over 24 h (liposome-encapsulated component).

Preclinical studies have confirmed favourable pharmacokinetic characteristics of inhaled liposomal ciprofloxacin. In animal models, the liposomal component has a lung clearance half life after inhalation of approximately 12 h[11] (compared with approximately 1 h for free ciprofloxacin)[12] supporting once-daily dosing. Additionally, inhalation of liposomal ciprofloxacin has demonstrated superior efficacy to unencapsulated ciprofloxacin in a murine model of lethal pulmonary *Francisella Tularensis* infection.[12] Human studies of DRCFI confirm a systemic half life of approximately 10 h and sputum ciprofloxacin concentrations persistently above 20 µg/g out to 22 h.[13] A comprehensive programme of phase I and II studies (comprehensively reviewed in[14]) suggest optimal pharmacokinetic properties and microbiological efficacy of the DRCFI formulation and dose evaluated in the current study.

The primary objective of ORBIT-2 (Once daily Respiratory Bronchiectasis Inhalation Treatment), a 24-week, phase II efficacy and safety study, was to evaluate the microbiological efficacy of 28 days of inhaled DRCFI. The total study duration of 24 weeks was selected to provide the opportunity to assess safety, tolerability and generate pulmonary exacerbation data. Given the increased risk of respiratory AEs demonstrated previously with inhaled therapies in non-CF bronchiectasis,[5–9] subjects reaching the pulmonary exacerbation endpoint were discontinued from further exposure to trial medication (but remained in the study) to mitigate any possibility of a similar experience with DRCFI.

METHODS

See the online data supplement for full details relating to all methods.

Subjects

Clinically stable adults with CT scan-proven bronchiectasis, *P aeruginosa* airway infection and ≥2 pulmonary exacerbations requiring antibiotic therapy in the preceding 12 months were eligible. Subjects with CF, bronchopulmonary aspergillosis or pulmonary non-tuberculous mycobacterial infection were excluded. At least one ciprofloxacin-sensitive *P aeruginosa* strain needed to be cultured from sputum during a 14-day screening period in order to proceed to randomisation. The study was approved by the ethics committee review at each study site and all subjects provided written consent.

Study design and procedures

This was a 24-week, multicentre, randomised, double-blind, placebo-controlled study of DRCFI undertaken in 11 sites in Australia and New Zealand. Eligible subjects were centrally randomised 1:1, stratified by number of pulmonary exacerbations (2–3 or ≥4) in the preceding 12 months. DRCFI consisted of liposomal ciprofloxacin for inhalation (150 mg in 3 ml) and free ciprofloxacin (60 mg in 3 ml), each provided in separate vials. Matched placebo consisted of control liposomes (15 mg in 3 ml) and normal saline (0.9%, 3 ml). Subjects nebulised DRCFI or matching placebo once-daily through a PARI LC Sprint powered by a PARI Turbo Boy-S compressor (PARI, Richmond, USA) for up to three treatment cycles of 28 days 'on' inhaled therapy, 28 days 'off' (figure 1; see online data supplement). Trial medication was discontinued once subjects reached the pulmonary exacerbation endpoint although subjects remained in the study and continued trial visits and assessments. Hence, all subjects (excepting subjects who withdrew from the study for AEs prior to experiencing pulmonary exacerbation) contributed to pulmonary exacerbation data.

Subjects were reviewed after 2, 4 and then every 4 weeks. Assessments performed at each visit included spirometry, sputum collection, 6 min walk test (6MWT) and St George's Respiratory Questionnaire (SGRQ). Sputum was collected 24 h postdose at the end of each 'ON' period and transferred at ambient temperature[15] by courier on the day of collection to a central laboratory (Dorevitch Pathology, Melbourne, Australia). Culture and identification of pathogens, *P aeruginosa* quantitative bacteriology and ciprofloxacin minimal inhibitory concentration measures of *P aeruginosa* isolates were performed.[15] For quantitative bacteriology, sputum was homogenised 1:1 with Sputasol (Oxoid, Basingstoke, UK) and serially diluted with sterile saline. The 10 µl samples were inoculated onto chocolate agar and incubated for 24 h. The colony forming unit (CFU) count from the lowest dilution plate containing 30–300 discrete colonies was recorded.

Outcomes

The primary efficacy variable was mean change in sputum *P aeruginosa* bacterial density (as $\log_{10}$ CFU/g of sputum) from baseline to the end of the first treatment cycle (day 28), comparing treatment with placebo and assessed on the full analysis set (all subjects who received at least one dose of study drug, hereafter modified intention to treat, mITT). Secondary outcome measures included time to first pulmonary exacerbation, forced expiratory volume in 1 s (FEV_1), 6MWT, SGRQ, safety and tolerability. Protocol-defined pulmonary exacerbation, using a modification of Fuchs criteria,[16] was defined as deterioration in at least four of the following nine symptoms or signs: sputum production (volume, colour, consistency or haemoptysis), dyspnoea, cough, fever, wheezing, exercise tolerance (or fatigue/lethargy/ malaise), FEV_1 or FVC fall of at least 10%, new changes on chest radiograph and changes in chest sounds on auscultation.

Data analysis

Using estimates of effect derived from pilot data (unpublished), 40 subjects would be needed to demonstrate a 4 $\log_{10}$ CFU/g (SD 3.5) difference between the active and placebo arms, with 90% power at the 0.05 significance level. The primary outcome was assessed on the mITT population by analysis of covariance with effects for annual pulmonary exacerbations as a blocking variable, the baseline value for *P aeruginosa* bacterial load as a covariate and the treatment effect. Per protocol analyses (defined as all randomised patients who did not experience major protocol deviations) were also performed. Time to pulmonary exacerbation was assessed by Kaplan–Meier survival curves. Given the trial design stipulating withdrawal from study drug following pulmonary exacerbation, data analysis for the majority of outcomes was prespecified on day 28 data although data were collected throughout the duration of the trial.

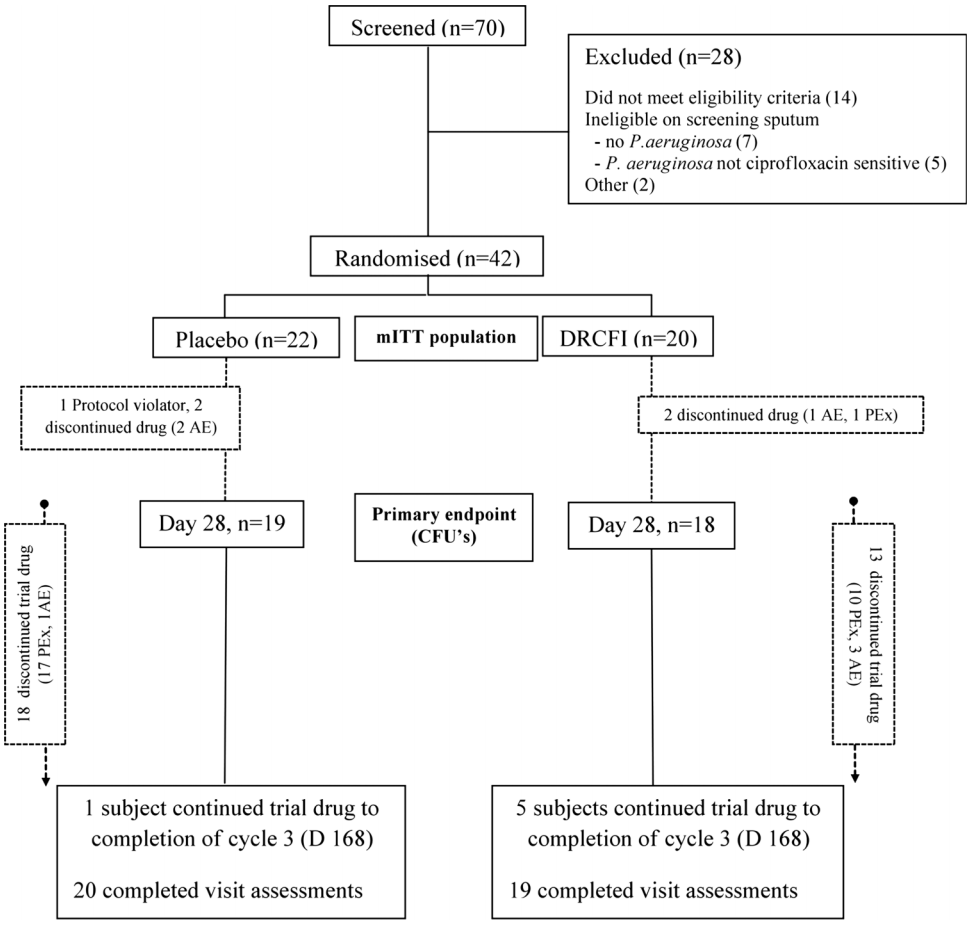

Figure 1 Trial flow diagram (AE, adverse event; CFUs, colony forming units; DRCFI, dual release ciprofloxacin for inhalation; mITT, modified intention to treat; PEx, pulmonary exacerbation; after randomisation, n refers to the number of subjects continuing to receive trial medication, although all subjects were encouraged to continue trial assessments until completion at day 168).

RESULTS

Between November 2009 and September 2010, 70 subjects were screened and 42 (22 placebo and 20 DRCFI) randomised (see figure 1 for trial flow and table 1 for patient demographics). A single subject was randomised in spite of not culturing *P aeruginosa* at screening in violation of the protocol. All 42 randomised subjects were included in the mITT analysis, and 39 (93%) completed assessments at the final visit on day 168.

DRCFI resulted in significant reductions in *P aeruginosa* bacterial density to day 28 compared with placebo (−4.2±3.7 vs −0.08±3.8 $\log_{10}$ CFU/g, p=0.002; figure 2). Limiting the analysis to the per protocol population showed a similar, statistically stronger, treatment effect (p<0.001). The reduction in bacterial density was seen early on, from the first visit at day 14. During each of the subsequent 'OFF' periods there was an increase in sputum *P aeruginosa* bacterial counts towards baseline. In spite of the retention of increasing numbers of subjects who were no longer inhaling active therapy as the trial progressed, mean reductions in bacterial counts were still seen in each of the subsequent DRCFI treatment ('ON') periods.

Pulmonary exacerbations were experienced by 17 (77%; all required antibiotic therapy) of placebo subjects and 11 (55%; 8 required antibiotic therapy) in the DRCFI group by day 168. Post hoc analysis revealed that the overall proportion of subjects requiring antibiotics for pulmonary exacerbation was lower in the DRCFI group (8 (40%) vs 17 (77%), OR 0.2, 95% CI 0.04 to 0.89, p=0.027 by Fisher's exact test). Intravenous antibiotics were received by three subjects in each group. The median time to pulmonary exacerbation by Kaplan–Meier analysis was 134 days for the DRCFI group and 58 days for the placebo group, although this achieved conventional statistical significance only on the per protocol population (p=0.057 mITT, p=0.046 per protocol, by log-rank test; see figure 3).

No significant differences were seen between the two treatment groups for changes to day 28 for other outcome measures including FEV_1 (DRCFI −0.05±0.12 vs placebo 0.00±0.10 L, p=0.18), SGRQ total score (DRCFI −1.3±7.16 vs placebo −6.4±9.8, p=0.08) or 6MWT distance (DRCFI 0.6±71.6 vs placebo −7.6±92.3 m, p=0.54).

Failure to culture *P aeruginosa* at day 28 was seen more frequently in the DRCFI than placebo arms (12 (60%) vs 3 (14%), OR 9.5, 95% CI 1.8 to 63.0, p=0.003 by Fisher's exact test). New sputum pathogens were cultured at any time during the study in 12 placebo subjects (55%) on 21 occasions, the most common organisms being *Stenotrophomonas maltophilia* (four subjects), *Pseudomonas fluorescens* (three) and *Streptococcus pneumoniae* (two). New sputum pathogens were cultured in 9 (45%) DRCFI subjects on 16 occasions, the most common organisms being *S maltophilia* (five), *Staphylococcus aureus* (three) and *Moraxella catarrhalis* (two).

The identification of *P aeruginosa* isolates with lowered categorical susceptibility to ciprofloxacin (according to CLSI breakpoints) at any time during the study occurred in eight placebo subjects (38%, seven intermediate susceptibility and one

Table 1 Baseline characteristics of the subjects

	Placebo (n=22)	DRCFI (n=20)
Age (years)*	59.5 (13.2)	70 (5.6)
Female—number (%)	13 (59.1)	10 (50)
FEV_1 (l)	1.47 (0.73)	1.57 (0.77)
FEV_1 per cent predicted (%)	53.1 (22.7)	60.7 (24.1)
Sputum *Pseudomonas aeruginosa* bacterial density (log_{10} CFU/g)	5.9 (2.7)	6.5 (2.3)
Other sputum organisms present in >1 subject†	Klebsiella spp. Ochrobactrum anthropi	Klebsiella spp.
Comorbidities—n		
Ischaemic heart disease	9	10
Hypertension	6	9
Diabetes mellitus	2	0
Cerebrovascular disease	1	0
Medications—n		
Maintenance oral macrolides	4	5
Inhaled corticosteroids	4	6
Combination inhalers (ICS/LABA)	9	11
Inhaled LABA	2	2
Inhaled SABA	13	10
Inhaled LA anticholinergic	5	7
Inhaled SA anticholinergic	4	1
Prednisone	1	1
Inhaled mannitol	2	2
Prior smokers—n	0	1

Values are mean (SD) except where otherwise indicated.
*p<0.01 for the comparison between groups for age; there were no significant differences between groups for other variables.
†n=2 for each of these organisms.
CFU, colony forming unit; DRCFI, dual release ciprofloxacin for inhalation, FEV_1, forced expiratory volume in 1 s; ICS, inhaled corticosteroids, LA, long-acting; LABA, long-acting β-agonists, SA, short-acting; SABA, short-acting β-agonists.

resistant) and 10 DRCFI subjects (50%, six intermediate, four resistant). The change in *P aeruginosa* ciprofloxacin minimal inhibitory concentrations to day 28 (evaluating the most resistant isolate identified) did not differ significantly between the groups (median (range) 0 (−0.5 to +31) vs 0 (−0.75 to +0.5), p=0.26 by Mann–Whitney U test).

DRCFI was well tolerated with the overall incidence of AEs similar to the placebo group. Serious treatment emergent AEs were reported for three subjects in each arm (all events were pulmonary exacerbations)—none of the DRCFI events were considered treatment-related. Respiratory-related AEs leading to study discontinuation were more frequent in the placebo group (13 vs 7 patients) while non-respiratory AEs leading to discontinuation were similar (three for placebo: one anal ulcer, one sinusitis, one skin graft infection; four for DRCFI: two nausea, one sinusitis, one fatigue). Treatment emergent AEs occurring in more than three subjects in either arm were: lung disorder (DRCFI 11 subjects, 55% vs placebo 19, 86%), product taste abnormal (DRCFI four subjects, 20% vs placebo zero), nausea (DRCFI four subjects, 20% vs zero) and headache (DRCFI one subject, 5% vs placebo four subjects, 18%).

DISCUSSION

In this phase II double-blind RCT, once daily, inhaled DRCFI demonstrated microbiological efficacy in non-CF bronchiectasis subjects with ciprofloxacin-sensitive *P aeruginosa* airway infection, without any evidence of tolerability or safety concerns. This microbiological efficacy, representing a greater than 10 000-fold fall in CFU (4 log fold drop), was associated with positive effects upon pulmonary exacerbation outcomes assessed by both time to first exacerbation (although statistically significant only in the per protocol population) and need for supplemental antibiotics. This is the first double-blind, placebo-controlled study of an inhaled agent to demonstrate clinical benefits in subjects with non-CF bronchiectasis, although it must be recognised that this was a specific, selected group of *P aeruginosa*-infected non-CF bronchiectasis subjects and hence the study results are not more broadly generalisable.

While the effects upon the primary outcome measure in ORBIT-2 were large and unambiguous, the effects upon pulmonary exacerbation outcomes need to be considered in the light of potential study limitations. First, the modest sample size creates the risk of Type 1 error. Second, the time to exacerbation endpoint was only conventionally statistically significant on the per protocol population (p=0.057 on mITT). Third, the DRCFI group were older and we cannot exclude that random imbalance in subject age at baseline may have influenced exacerbation outcomes in the two groups. However, baseline pulmonary exacerbation rates (a stratification variable at randomisation)

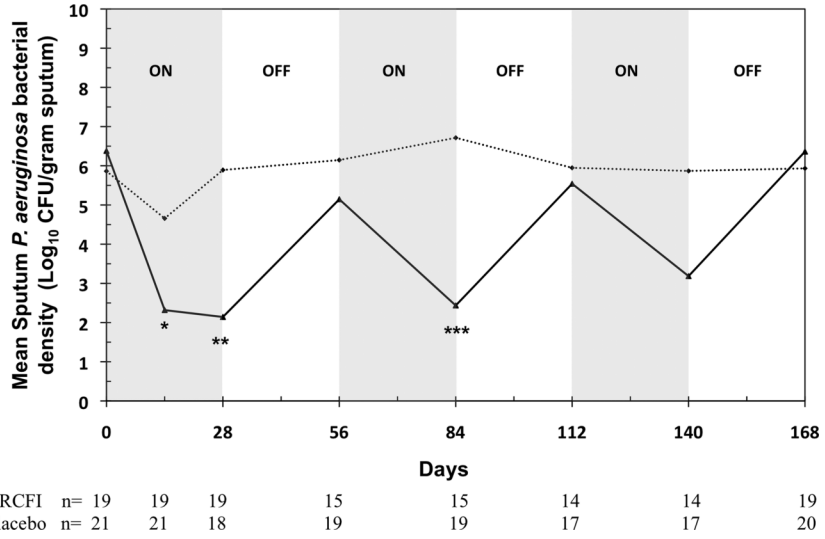

Figure 2 Change in mean sputum *Pseudomonas aeruginosa* bacterial density across the 24 weeks of the study comparing DRCFI and placebo groups in the modified intention to treat (mITT) population. (Dotted line represents placebo, solid line represents DRCFI; note that data presented here are from both subjects who remained on trial drug *and* those who had withdrawn from trial drug due to pulmonary exacerbation; *p<0.05, **p<0.01, ***p<0.001 comparing DRCFI and placebo groups for change in bacterial density from baseline; CFU, colony forming unit; DRCFI, dual release ciprofloxacin for inhalation.)

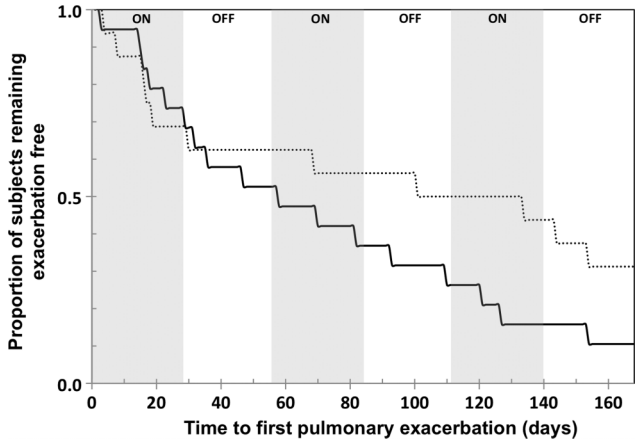

Figure 3 Kaplan–Meier curves comparing DRCFI and placebo groups for time to first pulmonary exacerbation in the modified intention to treat (mITT) population. (Dotted line represents DRCFI, solid line represents placebo; median 134 vs 58 days, p=0.057 mITT, p=0.046 per protocol, by log-rank test; DRCFI, dual release ciprofloxacin for inhalation.)

were matched. Finally, the novel trial design employed in the current trial, in which subjects were withdrawn from study drug following first exacerbation, did reduce the potential to capture longer-term safety and tolerability data in some patients. However, this design safeguarded patients (given that many prior studies of inhaled therapies in this subject group have shown increased respiratory AEs)[5–9] and yet ensured that the primary and key secondary (time to first pulmonary exacerbation) outcome measures were robustly captured. Furthermore, the potential duration of drug exposure in the current study was actually substantially longer than has been the norm in recent studies of inhaled antibiotics in both CF and non-CF bronchiectasis, which have mostly consisted of single 28-day antibiotic cycles.[6 17–21]

The antimicrobial efficacy of DRCFI developed early following commencement of therapy and was seen with each of the subsequent repeat treatment cycles. The reduction in bacterial density with DRCFI was similar in magnitude to that previously reported for TOBI in bronchiectasis subjects[6] and substantially greater than the 2.2 $\log_{10}$/g reduction seen with TOBI in CF subjects after 4 weeks of therapy.[4] However, in contrast to the prior study of nebulised TOBI in bronchiectasis,[6] in ORBIT-2 there were fewer respiratory-related AEs and reductions in exacerbations in subjects receiving DRCFI compared with placebo. The recently reported single-blind study of low-dose inhaled gentamicin[10] and double-blind RCT of inhaled ciprofloxacin dry-powder inhaler[17] have also both demonstrated reductions in bacterial density of a similar magnitude to that reported here. However, both of those studies assessed this outcome in a range of airway pathogens in contrast to ORBIT-2 which assessed *P aeruginosa* alone.

DRCFI was well tolerated, without bronchodilator premedication. It is unclear whether this simply reflects improved tolerability of ciprofloxacin generally compared with aminoglycosides, given that ciprofloxacin dry-powder inhaler was not associated with bronchospasm in a recent study,[17] or whether liposomal encapsulation may further improve tolerability.

Although there was no significant positive effect on quality of life to accompany the improvements in exacerbation data and respiratory AEs, this was not surprising as the study was not powered for this endpoint. Interestingly, two larger, recently reported studies of macrolides have also failed to show significant between-arm improvements in SGRQ scores in spite of significant improvements in a number of important clinical outcomes.[21 22]

In the current study, only subjects demonstrating at least one ciprofloxacin-sensitive *P aeruginosa* strain were enrolled and whether a significant benefit of therapy would emerge in subjects without any sensitive strains is unclear. However, we would anticipate that subjects with only resistant *P aeruginosa* strains on in vitro susceptibility testing will also derive clinical benefit from this formulation as DRCFI achieves high and sustained airway concentrations of ciprofloxacin that are likely to overcome even the most resistant strains.[13] Furthermore, in studies of TOBI in CF, nearly 30% of subjects had tobramycin-resistant *P aeruginosa* and these subjects demonstrated similar clinical benefits to those with fully susceptible strains.[4] Future studies evaluating the clinical efficacy of DRCFI in those without ciprofloxacin-sensitive *P aeruginosa* are needed.

The potential for development of antimicrobial resistance is inherent to any antibiotic therapy, particularly when used as a chronic maintenance therapy. While the current study did not demonstrate any significant increased resistance of *P aeruginosa* isolates to ciprofloxacin, some degree of selection pressure will result from the long-term use of any inhaled antibiotic including ciprofloxacin. For individuals with non-CF bronchiectasis, the critical question is whether the long-term use of inhaled antibiotics (including DRCFI) will result in improvements in clinical outcomes that outweigh the risks related to reductions in antimicrobial susceptibility. Only adequately powered long-term studies will address this question, although the data in subjects with CF suggest that the benefits of long-term nebulised antibiotics far outweigh any negative outcomes resulting from resistance induction.[4 23] Until there are data to further inform this dilemma, it would be prudent to limit consideration of inhaled antibiotic therapies in non-CF bronchiectasis to those subjects experiencing the greatest morbidity, specifically *P aeruginosa*-colonised individuals with frequent infective exacerbations (ie, the population studied in ORBIT-2).

One further potential limitation of a strategy of maintenance, cycled, inhaled ciprofloxacin for bronchiectasis relates to the lack of an alternative, orally active antipseudomonal antibiotic class. Put simply, if a patient with bronchiectasis develops an infective exacerbation while on inhaled ciprofloxacin, would there be any added benefit in treatment with an oral fluoroquinolone or will subjects have an increased risk of subsequently requiring intravenous (non-fluoroquinolone) antibiotics for rescue? The current study was not powered to assess this. Future studies will evaluate this by determining whether subjects inhaling DRCFI who exacerbate recover more slowly with oral antipseudomonal therapy or are more likely to require rescue with intravenous antibiotics.

In the current study, once-daily inhaled DRCFI was well tolerated and demonstrated potent antipseudomonal microbiological efficacy in adults with non-CF bronchiectasis and airway infection by ciprofloxacin-sensitive *P aeruginosa*. Furthermore, positive effects upon pulmonary exacerbation outcomes were seen for the first time in a double-blind RCT of inhaled antibiotics in this patient group, although this was statistically significant only per protocol and may have been influenced by baseline age imbalance in this modest-sized phase II study. Confirmatory results in a larger data set are now required, which will also inform upon the competing risk of antimicrobial resistance.

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Contributors IG, PB, DC, DJS, DB and ADS participated in study design; DJS, PJT, JK and HG recruited study subjects and conducted study procedures; PB, IG, DC and DJS analysed and interpreted the results; DJS drafted and wrote the paper; all authors reviewed and revised the paper. All authors approved the final version.

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Ethics approval Multicentre study—approved by ethics committees at each site.

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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.

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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)

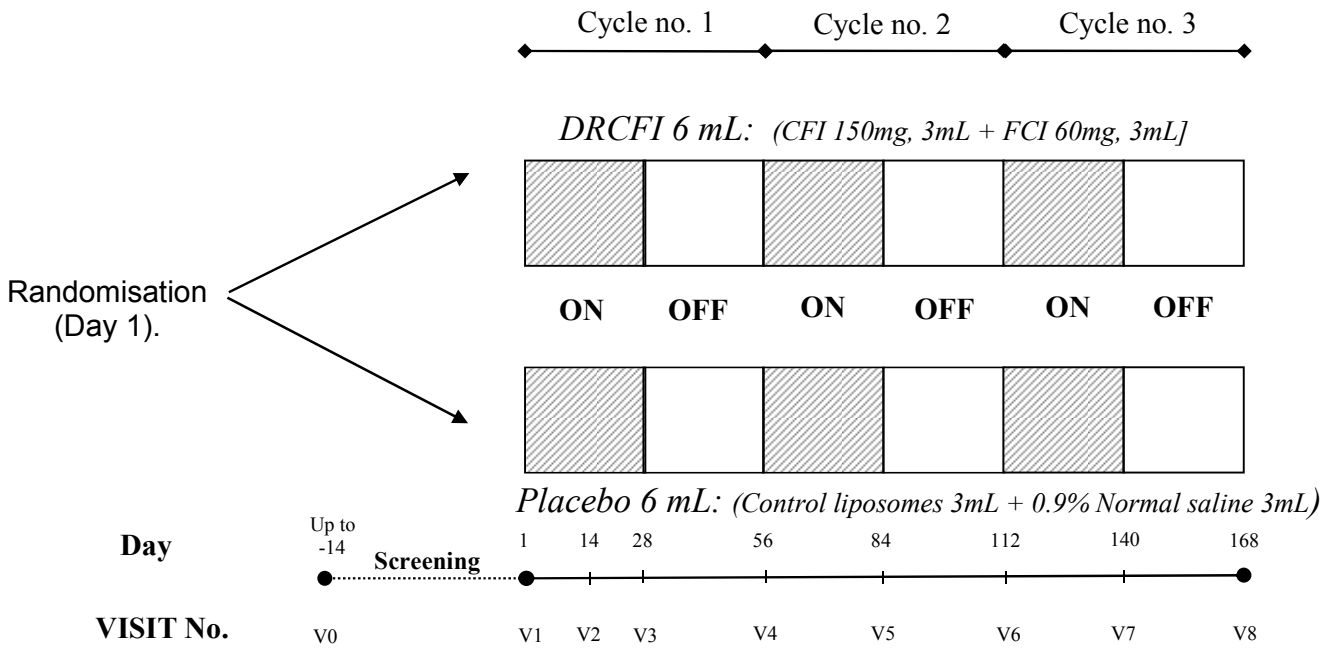
Cycle no. 1
Cycle no. 2
Cycle no. 3
DRCFI 6 mL: (CFI 150mg, 3mL + FCI 60mg, 3mL]
Randomisation
(Day 1).
ON
OFF
ON
OFF
ON
OFF
Placebo 6 mL: (Control liposomes 3mL + 0.9% Normal saline 3mL)
Day
Up to
-14
Screening
1
14
28
56
84
112
140
168
VISIT No.
V0
V1
V2
V3
V4
V5
V6
V7
V8

Figure 1.