Supplementary online material

Ciprofloxacin DPI in non-cystic fibrosis bronchiectasis: a phase II randomised study

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Materials and methods

Study subjects

Diagnostic restrictions were imposed to avoid pathologies that might affect the primary outcome measure (e.g. hypogammaglobulinaemia and allergic bronchopulmonary aspergillosis). To ensure sufficient disease severity, inclusion into the study required a history of exacerbations, with two or more courses of systemic antibacterials or one or more hospitalisation for intravenous antibacterial treatment for pulmonary exacerbations in the previous 12 months.

Prior to amendment 3 (dated 7 January 2010) of the protocol, a high number of sputum samples were rejected due to the strict epithelial cell requirement; therefore, some subjects were excluded from the study because of inadequate criteria for the limits of epithelial cell count per 100-times magnification field. After amendment 3, sputum samples were deemed adequate for microbiological analysis if they had ≤25 epithelial cells per field of view on microscopic examination (at 100-times magnification) and no leukocytes.

Definitions of analysis sets

Modified intent-to-treat population: all subjects who were randomised and who received at least one dose of study drug or placebo.

Per-protocol population: all subjects were included if: they had a confirmed diagnosis of non-cystic fibrosis bronchiectasis; they had a sputum culture positive for at least one of the pre-defined respiratory pathogens; no other systemic or inhaled antibacterial was administered between 4 weeks prior to randomisation and the end of treatment (EOT), apart from macrolides used as stable maintenance treatment for bronchiectasis for at least the last 30 days prior to randomisation; the study drug was taken with compliance of ≥80% (subjects who discontinued due to an exacerbation were included, provided their compliance was ≥80% before exacerbation); there was no protocol violation affecting the efficacy analyses; EOT

culture results were available; and if there was no co-existing disease affecting clinical or bacteriological outcomes.

Safety: all subjects who received at least one dose of study drug and with at least one observation on treatment were included in the safety analysis set.

Interim analysis

The interim analysis was conducted to assess whether the study should be discontinued either in case of apparent inefficacy of the study medication (ciprofloxacin dry powder for inhalation [DPI]) or in case the efficacy of the study medication was unexpectedly high. Safety (with particular attention to bronchospasm) was also considered. The interim analysis was performed by an independent data monitoring committee, which was composed of two pulmonologists with expertise on the disease state. The data for the analysis were prepared by an external third-party statistician (Axio Research Seattle, Washington, US), who was unblinded for this purpose.

Seventy subjects were calculated to provide a ≤10% probability that the study would be stopped on account of the reduction in bacterial load being at least 0.6 $\log_{10}$ colony-forming units (CFU) (stopping for futility) and >90% power when the true reduction of bacterial load would be >2 $\log_{10}$ CFU (stopping for unexpectedly high efficacy; expected drop 1.2 $\log_{10}$ CFU]; standard deviation 2.0). The number chosen also needed to be small enough to ensure that a decision on futility could be made in time to avoid new subjects receiving unnecessary treatment.

Study endpoints

Secondary endpoints included: time to exacerbation; emergence of new potential pathogenic microorganisms; emergence of resistance among baseline pathogens (defined as an increase in minimum inhibitory concentration to ciprofloxacin of >4 mg/L); microbiological response rate of ciprofloxacin DPI; changes in inflammatory biomarkers (including high-sensitivity C-reactive protein and absolute neutrophil count); and change in 24-hour sputum volume and colour from baseline. Routine laboratory tests (i.e. haemogram, renal and hepatic function tests) were performed and the incidence of abnormal findings in physical examinations and laboratory test results recorded. Changes from baseline in pulmonary function (forced expiratory volume in 1 second and forced vital capacity) and in health status (St George's Respiratory Questionnaire [SGRQ]) at EOT, 28 days after EOT (Day 56) and 56 days after EOT (Day 84) were also evaluated, in addition to adverse events and results of physical examinations, vital signs and laboratory analyses.

Exacerbations were defined using the parameters set by the American Thoracic Society/European Respiratory Society taskforce [1]

Statistical analysis

Sample size estimation was based on a difference of 1.2 $\log_{10}$ CFU/g, with standard deviations of 2.0 $\log_{10}$ CFU/g. The assumptions were based on conservative estimates from published results and included an adjustment for multiple testing. Calculations were made using ADDPLAN Version 4.0.3 (ADDPLAN GmbH, Cologne, Germany). Using α=2.3% (one-sided) and β=12.0%, the sample size estimation yielded n=61 subjects per treatment group as valid for modified intent-to-treat analyses. For calculation of sample size, NQuery Advisor® Version 6.01 module MTTO-1 was used.

Note: if the first dose of study drug was not administered on the date of randomisation and baseline procedures because of organisational reasons, Day 1 was defined as the first day of study drug administration.

Results – modified intent-to-treat analysis set

An overview of exacerbations that occurred during the study is presented in table S1. Because fewer than 50% of subjects had an exacerbation, the median time to exacerbation was not calculable.

Three subjects had an exacerbation requiring systemic antibacterial therapy but these subjects were not discontinued. All three completed the treatment period prior to receiving the first dose of antibacterial therapy: on Days 28 and 47 for the two subjects in the ciprofloxacin DPI group, and on Day 45 for the subject in the placebo group. Culture results for all three subjects after Day 28 were invalidated in both the modified intent-to-treat and per-protocol populations.

Results – per-protocol analysis set

Treatment compliance and duration

Compliance of ≥80% was observed in all subjects in both treatment groups (37/37 in the ciprofloxacin DPI group and 45/45 in the placebo group, respectively). The mean treatment duration in the per-protocol population was 27.8 days and 26.7 days for the ciprofloxacin DPI and placebo groups, respectively.

Secondary endpoints

Fewer subjects in the ciprofloxacin DPI group than in the placebo group reported exacerbations throughout the study (15 and 21 subjects, respectively). Of these, eight subjects in the ciprofloxacin DPI group required intervention with antibacterial therapy, including two subjects who required hospitalisation, compared with 15 subjects in the placebo group who required antibacterial therapy of whom four subjects required hospitalisation.

Eradication (negative bacterial culture; only results from valid cultures that were positive at baseline were considered) was observed in 46% (13/28) of subjects in the ciprofloxacin DPI group compared with 10% (4/40) of subjects in the placebo group at Day 8, and in 38% (12/32 ciprofloxacin DPI) of subjects and 7% (3/41 placebo) of subjects at EOT. The appearance of a new potential respiratory pathogen during treatment occurred less frequently in subjects receiving ciprofloxacin DPI than in those in the placebo group (cumulative frequency: 9 and 21 subjects, respectively).

The change in sputum colour for the per-protocol population is shown in table S2.

Mean high-sensitivity C-reactive protein values were numerically lower for the ciprofloxacin DPI group at EOT than for the placebo group (8.81 mg/L vs 16.17 mg/L, respectively; p=0.173). Evidence for a treatment effect on absolute neutrophil count was indicated by a significant reduction at EOT for ciprofloxacin DPI compared with placebo (4.85×10^6/mL vs 6.07×10^6/mL, respectively; p=0.010).

The values for the mean SGRQ scores at baseline, EOT, 4-week follow-up and end-of-study visits are summarised in table S3, for both the modified intent-to-treat and per-protocol populations.

Table S1. Overview of exacerbations

	Ciprofloxacin DPI	Placebo
Number of subjects with exacerbations reported as AEs (number of events)		
All AEs of exacerbation	22 (25)	26 (29)
Treatment-emergent exacerbations*	7 (7)	14 (15)
Late exacerbations	17 (18)	13 (14)
All exacerbations leading to premature discontinuation of treatment	3[#]	6
Number of subjects with exacerbations reported as efficacy		
All	22	25[¶]
Requiring antibacterial treatment	14	18
Requiring hospitalisation	2	5

AE: adverse event; DPI: dry powder for inhalation.
* Treatment-emergent exacerbations defined as exacerbations occurring after the start of treatment up until 7 days after the end of treatment
[#] One subject had an exacerbation and received treatment but was not discontinued (this patient is shown Figure 4 of the main manuscript as having an exacerbation during treatment)
[¶] In one exacerbation in the placebo group, the Investigator reported this as an adverse event only

Table S2. Sputum colour in per-protocol population

	Ciprofloxacin DPI n (%)	**Placebo n (%)**
Baseline	N=37	N=45
No sputum/clear	3 (8.1)	3 (6.7)
Yellow	19 (51.4)	18 (40.0)
Green	15 (40.5)	21 (46.7)
Rust	0	3 (6.7)
End of treatment	N=35	N=39
No sputum/clear	9 (25.7)	3 (7.7)
Yellow	22 (62.9)	20 (51.3)
Green	3 (8.6)	16 (41.0)
Rust	1 (2.9)	0
4-week follow-up	N=29	N=28
No sputum/clear	7 (24.1)	3 (10.7)
Yellow	11 (37.9)	16 (57.1)
Green	8 (27.6)	8 (28.6)
Rust	3 (10.3)	1 (3.6)

DPI: dry powder for inhalation.

Table S3. St George's Respiratory Questionnaire total mean scores (SD) in modified intent-to-treat and per-protocol populations

	Ciprofloxacin DPI N=60 Mean (SD)	**Placebo N=64 Mean (SD)**
Modified intent-to-treat population		
Baseline	43.79 (20.33)	44.72 (18.06)
End of treatment	41.47 (21.03)	44.79 (19.81)
4-week follow-up	40.59 (20.89)	44.11 (18.55)
8-week follow-up	40.58 (18.13)	41.56 (16.99)
	Ciprofloxacin DPI N=37 Mean (SD)	**Placebo N=45 Mean (SD)**
Per-protocol population		
Baseline	48.81 (19.17)	43.59 (18.37)
End of treatment	44.51 (20.71)	43.92 (19.26
4-week follow-up	42.85 (20.70)	43.99 (18.37)
8-week follow-up	44.49 (16.24)	42.84 (17.42)

DPI: dry powder for inhalation; SD: standard deviation.

Reference

1. Cazzola M, MacNee W, Martinez FJ, Rabe K, Franciosi LG, Barnes PJ, Brusasco V, Burge PS, Calverley P, Celli B, Jones PW, Mahler DA, Make B, Miravitlles M, Page C, Palange P, Parr D, Pistolesi M, Rennard SI, Rutten-van Molken MP, Stockley R, Sullivan SD, Wedzicha JA, Wouters EF. Outcomes for COPD pharmacological trials: from lung function to biomarkers. *Eur Respir J* 2008; 31: 416–468.