Phase II studies of nebulized Arikace® in CF patients with *Pseudomonas aeruginosa* infection

Authors: *J.P. Clancy[1], *L. Dupont[2], M.W. Konstan[3], J. Billings[4], S. Fustik[5], C.H. Goss[6], J. Lymp[7], P. Minic[8], A.L. Quittner[9], R.C. Rubenstein[10], K.R. Young [11], L. Saiman[12], J.L. Burns[6], J.R.W. Govan[13] B. Ramsey[6], and R. Gupta[14]; for the Arikace® Study Group

Affiliations: [1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [2]Katholieke Universiteit Leuven, Leuven, Belgium; [3]Case Western Reserve University, Cleveland, OH, USA; [4]University of Minnesota, Minneapolis, MN, USA; [5]University Clinical Centre, Skopje, Macedonia; [6]University of Washington, Seattle, WA, USA; [7]Seattle Children's Research Institute, Seattle, WA, USA; [8]Institute for Mother and Child Healthcare, Belgrade, Serbia; [9]University of Miami, Miami, FL, USA; [10]The Children's Hospital of Philadelphia and Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA; [11] University of Alabama at Birmingham; [12]Columbia University, New York, NY, USA; [13]University of Edinburgh, UK; [14]Insmed Incorporated, Monmouth Junction, NJ, USA

*Contributed equally to the conduct of this study and the content of this manuscript.

Supplemental Methods

Study agent

Arikace® consisted of single-use 5ml vials of neutral DPPC and cholesterol liposomes complexed with amikacin sulfate diluted in 1.5% saline (70mg/ml). Placebo was 1.5% saline without liposomes or amikacin.

Subjects

Patients and families were approached for participation by members of the local study teams at the CF care centers. Consent or assent (for minors) was obtained based on local IRB practice and approvals. All patients and/or families provided written informed consent to participate in the study. Patients could continue with other chronic pulmonary therapies (including hypertonic saline, azithromycin, inhaled corticosteroids, bronchodilators, and dornase alpha). Those on chronic inhaled antibiotics were to refrain from use for the duration of the study unless they developed new symptoms or reduction in pulmonary functions that in the opinion of their treating physician required treatment with inhaled antibiotics. Exclusion criteria included infection with *Burkholderia cepacia* or nontuberculous mycobacteria, active allergic bronchopulmonary aspergillosis, or significant liver, kidney, or other organ disease that in the opinion of the onsite investigator could place the patient at risk for complications related to study participation.

A total of 112 subjects were enrolled and 105 were dosed (Figure 1). Reasons for screen fails included: FEV_1, 40% (exclusionary criterion), pulmonary exacerbation in the exclusionary window; acute changes on chest X-ray, lack of the numbers of required *Pseudomonas aeruginosa* cultures in the prior two years (protocol required four positive cultures); refusal of consent for PK evaluation which was mandatory in the study; inability to withhold inhaled

antibiotic in the off month; requiring staph antibacterial suppressive treatment; or exclusionary clinical laboratory values. Four subjects developed pulmonary exacerbations during the washout period, one developed renal function exclusionary criteria, one withdrew consent, and one pediatric subject was withdrawn by parent (after randomization and prior to Day 1 dosing).

Secondary endpoints

Secondary objectives included centrally performed sputum microbiology, *P. aeruginosa* sputum density, and MIC_{50} and MIC_{90} of isolates for amikacin. Additional secondary endpoints included spirometry [Forced Expiratory Volume in one second (FEV_1), Forced Expiratory $Flow_{25–75\%}$ ($FEF_{25-75\%}$), and Forced Vital Capacity (FVC)], need for rescue antibiotics for pulmonary exacerbations, and change in Respiratory Symptoms on the CFQ-R (Quittner AL, Modi AC, Wainwright C, Otto K, Kirihara J, Montgomery AB. Determination of the minimal clinically important difference scores for the cystic fibrosis questionnaire-revised respiratory symptom scale in two populations of patients with cystic fibrosis and chronic pseudomonas aeruginosa airway infection. *Chest* 2009;135(6):1610-1618); Modi AC, Quittner AL. Validation of a disease-specific measure of health-related quality of life for children with cystic fibrosis. *Journal of Pediatric Psychology* 2003;28(8):535-45; Quittner AL, Buu A, Messer MA, Modi AC, Watrous M. Development and validation of The Cystic Fibrosis Questionnaire in the United States: a health-related quality-of-life measure for cystic fibrosis. *Chest* 2005;128(4):2347-2354). Pulmonary exacerbations were defined using the Fuchs criteria (Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, Rosenstein BJ, Smith AL, Wohl ME. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. *The New England Journal of Medicine* 1994;331(10):637-642).

Microbiology Methods

Sputum specimens were transported via special couriers, maintaining cold chain, to the Edinburgh Central Microbiology Laboratory of Professor Govan, to be plated within 48 hours of obtaining specimen. A similar transportation mechanism was used for US specimens delivered to the Central Laboratory of Professor Jane Burns at Seattle Children's Hospital. A harmonized protocol (on file at Insmed) using methods previously described (Isenberg, Henry, D., Editor. 1992. Clinical Microbiology Procedures Handbook from the American Society for Microbiology, Washington, D.C., Section 1.5 and 13 CHRMC Microbiology QC manual; Murray, Patrick R. 2003. 8th Edition. Chapters XX and XXI. Manual of Clinical Microbiology, American Society for Microbiology, Washington, D.C.; Burns, J. L., J. M. Van Dalfsen, R. M. Shawar, K. L. Otto, R. L. Garber, J. M. Quan, A. B. Montgomery, G. M. Albers, B. W. Ramsey, and A. L. Smith. 1999. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. J. Infect. Dis. 179:1190–1196; Wong, K., M.C. Roberts, L. Owens, M. Fife, AL Smith. 1984. Selective media for the quantitation of bacteria in cystic fibrosis sputum. J Med Microbiol. 17(2):113-1190) was used by both labs; for CF sputum inspection, weight/volume, processing with sputolysin, culturing on specific agar, *P. aeruginosa* identification, and quantitative methodology using serial dilutions and plating were employed. Specimens not meeting the criteria of minimum volume of 0.4ml and available at laboratory within 48h for culture were rejected.

Study design – open label extension

Following a review of the safety and pharmacokinetic data, an open label extension study of repeated Arikace® cycles was conducted at the European sites, utilizing a dosing schedule of 28 days of once daily Arikace® (560mg) followed by 56 days off treatment (six cycles). All

subjects enrolled in the extension study had been enrolled in the 28d placebo-controlled trial (n=49).

Pharmacokinetic Analysis

Blood samples were collected predose, at 0-1h and 3-4h post dose on Day 1, and predose and at 0-1h post final dose on Day 28 to perform PK analysis. In addition, blood samples were taken on Day 35. Sputum samples were obtained one hour post dosing on Days 1, 14, and 28.

Concentrations of amikacin in serum were determined by a sensitive and specific liquid chromatography method with tandem mass spectrometry detection (LC-MS/MS) with a lower limit of quantification of 0.15mg/L and an inter-day CV% of <6.95%. Concentrations of amikacin in sputum were determined by a similar tandem mass spectrometry detection method (LC-MS/MS) with a lower limit of quantification of 0.1mg/L and an inter-day CV% of 8.6%. Concentrations in the sputum were then corrected based on the weight of the sample to obtain sputum concentrations in µg/g.

Statistical analysis.

The effect of Arikace® on lung function and *P. aeruginosa* sputum density were determined by repeated measures ANOVA. Analyses of the CFQ-R scales included calculating both the absolute (Study day value – Day 1 value) and relative changes; scores for each domain were summarized by visit. The absolute change in domain score was calculated between Days 1-15, 15-28, and 28-42, and then analyzed by ANCOVA. Day 1 (baseline) scores were used as the covariate. The MID score for the CFQ-R Respiratory scale in patients chronically infected with *P. aeruginosa* (four points [Quittner AL, Modi AC, Wainwright C, Otto K, Kirihara J, Montgomery AB. Determination of the minimal clinically important difference scores for the cystic fibrosis questionnaire-revised respiratory symptom scale in two populations of patients

with cystic fibrosis and chronic pseudomonas aeruginosa airway infection. *Chest* 2009;135(6):1610-1618]) was summarized for changes from Day 1 to Day 15, and Days 15-28. A responder analysis, categorizing patients into three groups based on the MID (improved – increase >4 points; stable - change of <±4 points; worsened – decrease >4 points), was conducted using the relative change scores for each assessment period. Missing CFQ-R data from Day 28 (three Arikace® 560mg subjects, one placebo) were categorized as 'stable' relative to prior measurements. Treatment groups within each cohort were compared using chi-square tests. Additional supportive analyses included Pearson correlation coefficients between changes in CFQ-R Respiratory Symptoms and changes in FEV_1 % predicted from baseline values in the 560mg vs placebo groups.

Supplemental Results

Patient-Reported Outcomes

Results from the Respiratory domain of the CFQ-R indicated that at Day 28 of treatment, 24 (66.7%) of the 560mg dose group reported clinically significant improvements (MCID increase ≥4 points), compared to 13 (36.1%) of placebo-treated patients (Figure S2, *P*=0.006). There were no significant differences observed in the lower Arikace® dose groups or in other CFQ-R domains (Quittner AL, Modi AC, Wainwright C, Otto K, Kirihara J, Montgomery AB. Determination of the minimal clinically important difference scores for the cystic fibrosis questionnaire-revised respiratory symptom scale in two populations of patients with cystic fibrosis and chronic pseudomonas aeruginosa airway infection. *Chest* 2009;135(6):1610-1618). The differences in Respiratory Symptom scores between the 560mg Arikace® and placebo group was statistically significant (*P*=0.015). Changes in the CFQ-R Respiratory domain also

correlated with changes in FEV_1 % predicted across the dose groups at Day 14 (r=0.26, *P*=0.04), Day 28 (r=0.42, *P*=0.0006), and Day 42 (r=0.34, *P*=0.009).

Pharmacokinetic/Pharmacodynamic Results

Amikacin pharmacokinetics in sputum, serum, and urine are summarized here and in Table S3. This data is presented as median (range) due to non-normal distribution. For the 560mg dose group, median sputum concentrations of amikacin 1h post-dosing (min, max) on Day 1 were 2286mcg/gm (11.6, 11220), on Day 14 were 2187mcg/gm (5.79, 13014), and on Day 28 were 1758mcg/gm (8.28,15109). Pre-dose values on Day 14 were 35.9mcg/gm (2.17, 906) and on Day 28 were 41.1mcg/gm (3.29, 452), indicating that the airway clearance of amikacin was consistent over the 28-day dosing cycle. Mean serum amikacin C_{max} values were 1.29mcg/L (±0.77, SD) on Day 1 in patients dosed with 560mg, with a slight increase to 2.40mcg/L (±1.62) by Day 28 or dosing. T_{max} values also remained consistent across the trial, varying between 1-3h in a fashion that was not clearly dependent on dose. The urine detection of amikacin increased in a dose-dependent fashion and also increased by 25-50% over the course of the 28-day dosing cycle within the dose groups. Pharmacodynamic analyses demonstrated small but consistent and significant correlations between lung function [FEV_1 (absolute change, and the change in % predicted) and $FEF_{25\%-75\%}$] and dose at Days 7, 14, 21, and 28 of treatment (the range of r^2 values over these time points were 0.042-0.136; with *P* values ranging from *P*<0.001 to *P*=0.05).

Supplemental Tables

Table S1. Demographic information of subjects enrolled in placebo-controlled European trial.

	Arikace 280mg (n=21)	Arikace 560mg (n=21)	Placebo (n=22)
Age (yr)	16.0 (5.3)	16.6 (6.1)	17.0 (6.8)
Females, n (%)	16 (76.2%)	11 (47.8%)	12 (54.5%)
FEV_1 (L)	2.022 (0.788)	1.937 (0.936)	1.97 (0.65)
FEV_1 (% predicted)	66.4 (20.0)	62.9 (18.2)	68.0 (22.4)
$FEF_{25-75\%}$ (L/sec)	1.6 (0.9)	1.7 (1.0)	1.6 (1.0)
FVC (L)	2.8 (1.1)	2.6 (1.3)	2.7 (1.0)
BMI (kg/m^2)	18.059 (2.286)	18.877 (3.815)	18.6 (3.3)

Table S2. Demographic information of subjects enrolled in placebo-controlled US trial.

	Arikace 70mg (n=7)	Arikace 140mg (n=5)	Placebo 70 & 140mg (n=7)	Arikace 560mg (n=15)	Placebo 560mg (n=7)
Age (yr)	33.1 (9.7)	35.4 (6.0)	24.4 (6.3)	31.5 (14.5)	26.3 (6.7)
Females, n (%)	6 (85.7%)	1 (20.0%)	5 (71.4%)	5 (33.3%)	3 (42.9%)
FEV_1 (L)	1.866 (0.413)	2.878 (0.401)	2.436 (0.576)	2.409 (0.780)	2.347 (0.884)
FEV_1 (% predicted)	59.286 (12.593)	70.400 (10.090)	69.286 (16.550)	68.800 (17.026)	66.143 (12.020)
$FEF_{25-75\%}$ (L/sec)	0.964 (0.520)	1.912 (0.924)	1.506 (0.664)	1.644 (0.884)	1.391 (0.764)
FVC (L)	3.003 (0.604)	4.252 (0.421)	3.694 (0.639)	3.454 (1.013)	3.613 (1.434)
BMI (kg/m^2)	22.969 (1.567)	26.333 (3.193)	21.094 (2.467)	22.452 (3.405)	22.817 (2.737)

Table S3. Severe (Grade 3) adverse events (pooled from both the European and US trials).

	Arikace 70mg (N=7) n (%)	Arikace 140mg (N=5) n (%)	Arikace 280mg (N=21) n (%)	Arikace 560mg (N=36) n (%)	Placebo (N=36) n (%)
Lung Disorder (pulmonary exacerbation)	0 (0)	1 (20)	0 (0)	3 (8)	2 (6)
Pyrexia	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Laryngitis	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Tooth Abscess	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Arthralgia	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Lymphocyte Count Decreased	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
White Blood Cell Count Decreased	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)

Table S4. Related adverse events (possibly or probably as assessed by onsite investigators; pooled from both the European and US trials).

	Arikace 70mg (N=7) n (%)	Arikace 140mg (N=5) n (%)	Arikace 280mg (N=21) n (%)	Arikace 560mg (N=36) n (%)	Placebo (N=36) n (%)
Hepatic Enzyme Increased	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)
Dysgeusia	0 (0)	0 (0)	1 (5	0 (0)	0 (0)
Bronchial Obstruction	0 (0)	0 (0)	1 (5	0 (0)	0 (0)
Cough	0 (0)	0 (0)	1 (5	1 (3)	0 (0)
Urticaria	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
Nausea	1 (14)	0 (0)	0 (0)	1 (3)	0 (0)
Pruritus	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Dyspnoea	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
Motion Sickness	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)
Wheezing	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)
Creatinine Renal Clearance	0 (0)	1 (20)	0 (0)	0 (0)	1 (3)
Haemoptysis	0 (0)	1 (20)	0 (0)	0 (0)	1 (3)
Productive Cough	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Tinnitus	0 (0)	0 (0)	0 (0)	1 (3)	1 (3)
Vertigo	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
Dizziness	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
Headache	0 (0)	0 (0)	0 (0)	1 (3)	1 (3)
Paraesthesia	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
Lung Disorder (pulmonary exacerbation)	0 (0)	0 (0)	0 (0)	1 (3)	1 (3)
Fatigue	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Laryngitis	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Dysphonia	0 (0)	0 (0)	0 (0)	3 (8)	0 (0)
Pharyngolaryngeal Pain	0 (0)	0 (0)	0 (0)	3 (8)	0 (0)
Prolonged Expiration	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Rhonchi	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Throat Tightness	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)
Tachycardia	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
Chest Discomfort	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)

Table S5. Summary of all adverse events reported in 280mg, 560mg, and placebo treated subjects from the placebo-controlled trials.

	Arikace 70mg (N=7) n (%)	Arikace 140mg (N=5) n (%)	Arikace 280mg (N=21) n (%)	Arikace 560mg (N=36) n (%)	Placebo (N=36) n (%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.6%)	0 (0.0%)
LYMPH NODE PAIN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
LYMPHADENOPATHY	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
CARDIAC DISORDERS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
ARRHYTHMIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
TACHYCARDIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
EAR AND LABYRINTH DISORDERS	1 (14.3%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	3 (8.3%)
EAR CONGESTION	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
EAR PAIN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
MOTION SICKNESS	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
TINNITUS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	2 (5.6%)
VERTIGO	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
EYE DISORDERS	2 (28.6%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
CONJUNCTIVITIS	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
SCLERAL HYPERAEMIA	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
VISION BLURRED	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
GASTROINTESTINAL DISORDERS	2 (28.6%)	0 (0.0%)	0 (0.0%)	4 (11.1%)	6 (16.7%)
ABDOMINAL DISTENSION	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
ABDOMINAL PAIN	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
ABDOMINAL PAIN LOWER	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
ABDOMINAL PAIN UPPER	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
APHTHOUS STOMATITIS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
CONSTIPATION	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
DIARRHOEA	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	2 (5.6%)
NAUSEA	2 (28.6%)	0 (0.0%)	0 (0.0%)	2 (5.6%)	1 (2.8%)
STOMACH DISCOMFORT	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
VOMITING	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	3 (42.9%)	1 (20.0%)	1 (5.0%)	6 (16.7%)	8 (22.2%)
AXILLARY PAIN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
CHEST DISCOMFORT	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
CHILLS	0 (0.0%)	1 (20.0%)	0 (0.0%)	2 (5.6%)	1 (2.8%)
FATIGUE	2 (28.6%)	1 (20.0%)	0 (0.0%)	2 (5.6%)	1 (2.8%)
INFUSION SITE ERYTHEMA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
INFUSION SITE VESICLES	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
MUCOSA VESICLE	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
NON-CARDIAC CHEST PAIN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
PAIN	0 (0.0%)	1 (20.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
PYREXIA	0 (0.0%)	1 (20.0%)	1 (5.0%)	3 (8.3%)	3 (8.3%)
VESSEL PUNCTURE SITE HAEMATOMA	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
INFECTIONS AND INFESTATIONS	2 (28.6%)	0 (0.0%)	6 (29.0%)	9 (25.0%)	8 (22.2%)
BRONCHITIS	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
CHRONIC SINUSITIS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
INFLUENZA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)

	Arikace 70mg (N=7) n (%)	Arikace 140mg (N=5) n (%)	Arikace 280mg (N=21) n (%)	Arikace 560mg (N=36) n (%)	Placebo (N=36) n (%)
LARYNGITIS	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.6%)	0 (0.0%)
LOBAR PNEUMONIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
NASOPHARYNGITIS	1 (14.3%)	0 (0.0%)	1 (5.0%)	1 (2.8%)	2 (5.6%)
PHARYNGEAL CANDIDIASIS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
PHARYNGITIS	0 (0.0%)	0 (0.0%)	2 (10.0%)	0 (0.0%)	0 (0.0%)
PNEUMONIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
SINUSITIS	0 (0.0%)	0 (0.0%)	2 (10.0%)	2 (5.6%)	3 (8.3%)
TOOTH ABSCESS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
UPPER RESPIRATORY TRACT INFECTION	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
VIRAL INFECTION	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
SUNBURN	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
INVESTIGATIONS	2 (28.6%)	2 (40.0%)	0 (0.0%)	8 (22.2%)	3 (8.3%)
ALANINE AMINOTRANSFERASE INCREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
ASPARTATE AMINOTRANSFERASE INCREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
BLOOD ALKALINE PHOSPHATASE INCREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
BLOOD GLUCOSE DECREASED	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
BLOOD GLUCOSE INCREASED	1 (14.3%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
BLOOD URIC ACID INCREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
BREATH SOUNDS ABNORMAL	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
CREATININE RENAL CLEARANCE INCREASED	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
FORCED EXPIRATORY VOLUME DECREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
GAMMA-GLUTAMYLTRANSFERASE INCREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
HAEMATOCRIT INCREASED	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
HAEMOGLOBIN INCREASED	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
LIVER FUNCTION TEST ABNORMAL	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
NEUTROPHIL COUNT DECREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.6%)	0 (0%)
PLATELET COUNT INCREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
PROTEIN TOTAL INCREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
PULMONARY FUNCTION TEST DECREASED	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
WEIGHT DECREASED	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

	Arikace 70mg (N=7) n (%)	Arikace 140mg (N=5) n (%)	Arikace 280mg (N=21) n (%)	Arikace 560mg (N=36) n (%)	Placebo (N=36) n (%)
METABOLISM AND NUTRITION DISORDERS	0 (0.0%)	1 (20.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
DECREASED APPETITE	0 (0.0%)	1 (20.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
HYPERGLYCAEMIA	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	1 (14.3%)	0 (0.0%)	0 (0.0%)	6 (16.7%)	4 (11.1%)
ARTHRALGIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (8.3%)	1 (2.8%)
BACK PAIN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.6%)
MUSCLE SPASMS	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
MUSCLE TIGHTNESS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
MUSCULOSKELETAL CHEST PAIN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
MUSCULOSKELETAL PAIN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
MYALGIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
PAIN IN EXTREMITY	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
NERVOUS SYSTEM DISORDERS	0 (0.0%)	0 (0.0%)	3 (14.0%)	4 (11.1%)	5 (13.9%)
AMNESIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
DIZZINESS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	2 (5.6%)
HEADACHE	0 (0.0%)	0 (0.0%)	2 (10.0%)	2 (5.6%)	3 (8.3%)
MIGRAINE	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
PARAESTHESIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
SYNCOPE	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
PSYCHIATRIC DISORDERS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
DEPRESSION	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
INSOMNIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
RENAL AND URINARY DISORDERS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
POLLAKIURIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	6 (85.7%)	3 (60.0%)	8 (38.0%)	16 (44.4%)	14 (38.9%)
ASTHMA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
COUGH	1 (14.3%)	0 (0.0%)	2 (10.0%)	6 (16.7%)	4 (11.1%)
DYSPHONIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (8.3%)	0 (0.0%)
DYSPNOEA	0 (0.0%)	1 (20.0%)	0 (0.0%)	1 (2.8%)	2 (5.6%)
DYSPNOEA EXERTIONAL	1 (14.3%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
HAEMOPTYSIS	2 (28.6%)	1 (20.0%)	2 (10.0%)	1 (2.8%)	3 (8.3%)
LUNG DISORDER	2 (28.6%)	1 (20.0%)	1 (5.0%)	9 (25.0%)	6 (16.7%)
NASAL CONGESTION	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
NASAL MUCOSAL DISORDER	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
NASAL OEDEMA	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.6%)	0 (0.0%)
PHARYNGEAL ERYTHEMA	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
PHARYNGOLARYNGEAL PAIN	1 (14.3%)	0 (0.0%)	0 (0.0%)	3 (8.3%)	1 (2.8%)
PRODUCTIVE COUGH	2 (28.6%)	1 (20.0%)	3 (14.0%)	3 (8.3%)	5 (13.9%)
PROLONGED EXPIRATION	0 (0.0%)	1 (20.0%)	0 (0.0%)	2 (5.6%)	0 (0.0%)
PULMONARY CONGESTION	1 (14.3%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
RALES	0 (0.0%)	1 (20.0%)	0 (0.0%)	3 (8.3%)	0 (0.0%)
RESPIRATORY TRACT	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)

	Arikace 70mg (N=7) n (%)	Arikace 140mg (N=5) n (%)	Arikace 280mg (N=21) n (%)	Arikace 560mg (N=36) n (%)	Placebo (N=36) n (%)
CONGESTION					
RHINITIS ALLERGIC	0 (0.0%)	0 (0.0%)	2 (10.0%)	0 (0.0%)	0 (0.0%)
RHINORRHOEA	0 (0.0%)	1 (20.0%)	0 (0.0%)	3 (8.3%)	3 (8.3%)
RHONCHI	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (8.3%)	0 (0.0%)
SINUS CONGESTION	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	3 (8.3%)
SINUS DISORDER	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
SNEEZING	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
THROAT IRRITATION	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (2.8%)
THROAT TIGHTNESS	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (8.3%)	0 (0.0%)
WHEEZING	2 (28.6%)	0 (0.0%)	0 (0.0%)	3 (8.3%)	1 (2.8%)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.6%)	3 (8.3%)
DERMATITIS CONTACT	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
ERYTHEMA	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
HYPERKERATOSIS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
NIGHT SWEATS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
PHOTOSENSITIVITY REACTION	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
PRURITUS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
RASH	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)
URTICARIA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
SURGICAL AND MEDICAL PROCEDURES	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
SINUS OPERATION	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
VASCULAR DISORDERS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
FLUSHING	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)

Table S6. CTCAE grade change from baseline in audiology for subjects (pooled from both the European and US trials).

		Arikace 70mg (n=7) %	Arikace 140mg (n=5) %	Arikace 280mg (n=21) %	Arikace 560mg (n=36) %	Placebo (n=36) %
Day 28	None or minimal change	7 (100%)	3 (60%)	18 (85.7%)	30 (83.3%)	30 (83.3%)
	Grade 1	0 (0%)	2 (40%)	0 (0%)	0 (0%)	1 (2.8%)
	Grade 3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.8%)
Day 42	None or minimal change	6 (85.7%)	4 (80%)	18 (85.7%)	29 (80.6%)	29 (80.6%)
	Grade 1	0 (0%)	1 (20%)	0 (0%)	1 (2.8%)	2 (5.6%)
	Grade 3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.8%)

Table S7. Summary of adverse events in open label extension study of once daily Arikace® (560mg) over six treatment cycles (28 days of study drug, 56 days off study drug).

	All patients (n=49)
Number of adverse events	351
Number of possibly or probably related adverse events	33 (9.4%)
Patients with adverse events	48 (98%)
Patients with treatment-related adverse events	15 (30.6%)
Patients with Serious Adverse Events	15 (30.6%)
Patients interrupting study drug due to adverse events	1 (2%)
Deaths	0 (0%)

Table S8. Serum and urine pharmacokinetics of amikacin in Arikace®-treated subjects during the 28 day placebo-controlled trial, mean (SD) for European and US data combined.

Day	Dose (mg)	C_{max} (mg/L)	T_{max} (hr)	Total amikacin in 24 hr urine collection (mg)
1	70	0.22 (0.054)	2.05 (1.83)	4.79 (1.43)
	140	0.38 (0.17)	3.01 (1.92)	15.20 (8.74)
	280	0.95 (0.58)	1.05 (0.295)	17.70 (12.30)
	560	1.29 (0.77)	1.30 (1.29)	33.60 (26.80)
14	70	0.27 (0.060)	0.774 (0.31)	7.76 (3.19)
	140	0.45 (0.16)	2.81 (1.81)	21.70 (12.40)
	280	1.28 (1.02)	2.03 (3.75)	27.30 (16.50)
	560	1.95 (1.38)	1.80 (4.09)	46.20 (40.10)
28	70	0.29 (0.026)	1.57 (1.65)	7.13 (4.31)
	140	0.48 (0.21)	2.14 (1.79)	17.70 (8.33)
	280	1.42 (1.45)	0.812 (0.43)	25.20 (19.60)
	560	2.40 (1.62)	2.59 (5.60)	49.50 (46.10)

Supplemental Figure Legends

Figure S1. Arikace® enrollment in Europe and the US. Enrollment cohorts in the US at the beginning of the study included Placebo, 70mg, and 140mg. Following a DSMB and FDA review, enrollment in the 70mg and 140mg dose cohorts was closed, and continued in the 560mg and Placebo cohorts only.

Figure S2. Day 28 change in Respiratory Symptom domain scores (CFQ-R) in Arikace®-treated patients (560mg, n=36) compared with placebo controls (n=36). Data was pooled from the US and European studies. **LEFT**: 66.7% of Arikace®-treated subjects demonstrate improvement in MID (increase of ≥4) compared with 36.1% of placebo controls (P=0.009). **RIGHT:** 22.2% of Arikace®-treated subjects demonstrate reduction of MID (decrease of ≥4) compared with 38.9% of placebo controls ($P \leq 0.05$).

Figure S3. Median amikacin MIC_{90} of *Pseudomonas* per Arikace® 560mg treatment cycle. Each bar is the median amikacin MIC_{90} for *Pseudomonas* isolates at Days 1 and 28 of each Arikace® treatment cycle. The numbers below the abscissa are the study days and number of subjects providing data at each time point.